HIGH ABUNDANCE mtDNA RECOMBINANTS IN HETEROPLASMIC MICE: Implications for Assisted Reproduction and Therapeutic Cloning. *D.C. Wallace, M. Crimi.* Center for Molecular and Mitochondrial Medicine and Genetics (MAMMAG), University of California, Irvine, Irvine, CA 92697-3940.

To determine if mammalian mtDNAs can recombine when mixed in the same cytoplasm, we prepared mice using female ES cell cybrids that were heteroplasmic for the NZB and the Common inbred mtDNAs. These mtDNAs differ in 109 nucleotides (nt), including a BamHI polymorphism at nt 4277. The two mtDNAs were maintained in the heteroplasmic state through back crosses to C57BL/6 males. At generations 12 through 15, the proportion of the two mtDNAs was determined using the BamHI polymorphism, revealing three classes of mice: those which maintained more than 30%, less than 30%, and no detectable NZB mtDNAs. Heteroplasmy was confirmed in heart, brain, liver, testis, muscle, ovarian, kidney, though it was highest in liver. PCR amplification, cloning and sequencing of nt 3422 to 5552 from liver and ovary mtDNAs revealed that 4/9 liver and 1/7 ovary mtDNAs contained one of more crossovers between the NZB and Common mtDNAs. This extraordinary finding was confirmed by purifying the liver mtDNA from a heteroplasmic animal using two sequential CsCl-ethidium bromide gradients, amplification, cloning and sequencing and finding that 4 of 14 clones were recombinants, with the sequence shifting from the Common mtDNA to the NZB mtDNA in two distinct regions: nt 3600-4400 including ND1-3 tRNAs - ND2 and nt 8600-9400 encompassing COX III. A related analysis revealed recombinant molecules in 1/10 heart, 1/14 brain, 5/12 kidney, 2/12 muscle, 0/6 testis, and 4/14 ovarian clones. Somatic mutations were also observed. Therefore, recombinant mtDNAs can be prevalent in heteroplasmic mammals. These results are disturbing. In assisted reproduction, the cytoplasm from younger oocytes is being injected into older oocytes resulting in heteroplasmic children (Barritt, J., et al., 2001, Hum Repro 16:513) and in therapeutic cloning heteroplasmy can be expected. Consequently, both practices could result in recombinant mtDNAs, something that evolution has gone to considerable lengths to avoid. Supported from NIH grant NS21328 (DCW) and Dept. Neurological Sciences, University of Milan, fellowship (MC) .

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Development of a novel orally administered macrophage mediated gene therapy for Gaucher Disease. *E.I. Ginns, D.M. Faryna, M. Galdzicka, C. Chrzanowski, G.R. Ostroff.* Program in Medical Genetics, Pediatric Neurology, UMass Medical School, Worcester, MA 01605.

A novel orally administered macrophage delivered gene therapy is being developed for treatment of Gaucher disease. Current enzyme replacement therapy (ERT) improves blood counts and reverses hepatosplenomegaly. However, ERT is intravenously administered, costly, and has not significantly addressed bone or neurological complications. To evaluate the efficacy of our orally administered gene therapy method, DNA encoding human glucocerebrosidase (huGBA)was formulated inside micron-sized yeast cell wall particles (YCWP). YCWP-huGBA formulations were used to introduce huGBA DNA into J774 murine macrophages in culture and into long-lived Gaucher mice generated by gene targeting. As observed in human patients, the reduced GBA activity in these Gaucher mice results in glucocerebroside storage and Gaucher cells in tissues. The clinical manifestations in these mice can be accelerated by short courses of conduritol--epoxide. Following oral intake, YCWP-huGBA formulations are taken up through intestinal Peyers Patches where they are phagocytosed by macrophages that then migrate to organs of the reticuloendothelial system. Within the macrophage endosome the huGBA DNA is released at acid pH, migrates to the nucleus, and is expressed to produce normal huGBA. This macrophage mediated treatment results in huGBA expression in J774 murine macrophages *in-vitro,* and in tissues of Gaucher mice. Preliminary findings suggest that this therapy sufficiently corrects tissue GBA activity to ameliorate symptoms in treated, compared to untreated, severely affected Gaucher mice. As a consequence of improved delivery of huGBA, we expect that this approach will achieve significant reversal of tissue pathology, including bone. If macrophages containing huGBA migrate into brain, then resulting increased GBA levels could also provide clinical benefit for neurologic manifestations of the disease. The successful development of this therapy should provide a safer, more efficient and cost effective treatment for patients with Gaucher disease, as well as other lysosomal diseases.

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Adiponectin: An Inherited Survival Factor in Families with Exceptional Longevity. *A.R. Shuldiner[1], J. Crandall[2], T.I. Pollin[1], K. Tanner[1], M. Rincon[2], R. Lipton[2], N. Barzilai[2], G. Atzmon[2].* 1) University of Maryland School of Medicine,Baltimore; 2) Institute for Aging Research AECOM, New York.

Adiponectin (ADIPOQ), a serum protein expressed by adipose tissue, is lower with obesity and has a protective role against insulin resistance and atherosclerosis. To test the hypothesis that ADIPOQ influences survival, we examined ADIPOQ levels and genetic variation in the ADIPOQ gene in subjects with exceptional longevity. We studied unrelated Ashkenazi Jews between the ages of 60 and 108 years (n=366), of which 169 where over the age of 95 years. To test heritability, ADIPOQ levels were also measured in 222 of their offspring. Homozygosity for the ADIPOQ +2019 del/del increased from 12% at age 65 to 31% at age 105, (=0.35, p=0.05) and was associated with significantly higher serum ADIPOQ levels, independent of BMI (p = 0.01). Serum ADIPOQ levels were greater in subjects with exceptional longevity (13.00.5 ug/mL vs. 17.1 0.6 ug/mL in age 60-94 years and 95 years, respectively, p=0.0001), an effect that was independent of gender or BMI. Subjects with high serum ADIPOQ levels had a greater percentage of large HDL and LDL particle sizes(by NMR spectroscopy), higher HDL-cholesterol levels, lower insulin resistance (by HOMA) and lower prevalence of the metabolic syndrome (by NCEP III guidlines)compared to those with low ADIPOQ levels. The distribution of adiponectin levels in the offspring was bimodal, and ADIPOQ levels were significantly heritable (h2=0.36, p=0.05) in offspring, suggesting inheritance of a high (protective) ADIPOQ phenotype in families with exceptional longevity. Exceptional longevity is associated with high levels of ADIPOQ and with favorable lipoprotein profile and protection from the metabolic syndrome. This phenotype is inherited in part by a novel ADIPOQ genotype. We suggest that ADIPOQ may be an inherited survival factor in exceptional longevity acting by increasing insulin sensitivity and providing protection from the metabolic syndrome and cardiovascular disease.

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Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular, and cognitive development. *M.A. Tischfield[1,3], T.M. Bosley[4,5], M.A.M Salih[6], I.A. Alorainy[7], E.C. Sener[8], M.J. Nester[5], D.T. Oystreck[4], W. Chan[1], C. Andrews[1], R.P. Erickson[9], E.C. Engle[1,2,3].* 1) Dept of Medicine, Program in Genomics, Children's Hosp Boston; 2) Dept of Neurology, Children's Hosp Boston; 3) Program in Neuroscience, Div Med Sci, Harvard Med School, Boston; 4) Neuro-ophthalmology Div, King Khaled Eye Specialist Hospital, Riyadh, SA; 5) Neuroscience Dept, King Faisal Specialist Hospital and Research Centre, Riyadh, SA; 6) Division of Peds Neuro, Depart of Peds, College of Medicine, King Khalid Univ Hosp, Riyadh, SA; 7) Depart of Radiology & Diagnostic Imaging, College of Med, King Saud University, Riyadh, SA; 8) Dept of Ophthal, Hacettepe Univ Hospital, Ankara, Turkey; 9) Dept of Pediatrics, Mol. & Cell Biology, Univ. of Arizona College of Med, Tucson, AZ.

Using a positional cloning approach, we have identified homozygous coding mutations in HOXA1 in three genetically isolated populations in the Middle East and American southwest. Affected individuals have a pleiotropic spectrum of phenotypes including horizontal gaze abnormalities, facial weakness, deafness, hypoventilation, skull deformities, autism, mental retardation, internal carotid artery malformations, and conotruncal heart defects. Although horizontal gaze abnormalities and sensorineural deafness are the most penetrant aspects of the syndrome, the remaining phenotypes demonstrate considerable variable expressivity that can be dependent upon genetic background. This is the first report of mutations in a HOX gene critical for CNS development and the first description of viable homozygous mutations in any human HOX gene. The identified mutations, two non-sense and one frameshift, introduce premature stop codons in exon 1. Truncated mutant proteins should lack all known functional domains resulting in a complete loss of HOXA1 function. Our results demonstrate a new function for HOXA1 in vascular patterning that had not been reported in Hoxa1-/- mice. We also demonstrate that HOXA1 is necessary for proper cognitive and behavioral development, intriguing considering the canonical CNS expression domain of Hoxa1 has a rostal boundary in the brainstem.

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Ancient Hybridization in the History of *Homo sapiens*. *M.F. Hammer, D. Garrigan, Z. Mobasher, S.B. Kingan, J.A. Wilder.* Div Biotechnology, Univ Arizona, Tucson, AZ.

Fossil evidence links human ancestry with populations that evolved modern gracile morphology in Africa 130,000 - 160,000 years ago. Yet fossils alone do not provide clear answers to the question of whether the ancestors of all modern *Homo sapiens* comprised a single African population or an amalgamation of distinct archaic populations. DNA sequence data have consistently supported a single origin model in which anatomically modern Africans expanded and completely replaced all other archaic hominin populations. Here we present novel sequence data from a 17.5-kb X-linked locus, Xp21.1, that exhibits ancient divergence among lineages. We analyze levels of haplotype divergence and linkage disequilibrium (LD) in the framework of models predicting patterns of nucleotide variation expected as a consequence of admixture between historically isolated subpopulations. No previous human polymorphism study has been specifically designed to utilize these measures to assess the probability of a single population origin for anatomically modern humans. The Xp21.1 locus was selected because it is located in a non-coding region of the X chromosome with moderately high levels of recombination and very low gene density. This serves to minimize the potenital impact of natural selection acting on linked functional sites. Monte Carlo computer simulations show that it is highly improbable that the pattern of nucleotide variation observed at the Xp21.1 locus could arise in a single, panmictic population, as predicted by the recent African replacement model. We consider several plausible alternative hypotheses and conclude that ancient population structure in the evolutionary lineage leading to AMH is the most likely explanation for the Xp21.1 data. This inference supports human evolution models that incorporate admixture between divergent African branches of the genus *Homo*.

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The International HapMap Project: A haplotype map of the human genome with 4 million SNPs. *P. Donnelly, on behalf of The International HapMap Consortium.* University of Oxford, Oxford, UK.

The International HapMap Consortium, which includes scientists from Canada, China, Japan, Nigeria, the UK, and the US, has developed a human haplotype map describing the common properties and patterns of DNA sequence variation across the human genome. The primary data are the genotypes of about four million SNPs, their allele frequencies, and the degree of association among them, in a set of 270 DNA samples. In addition, ten 500 kb ENCODE regions were resequenced in 48 samples and the HapMap samples were genotyped for all known and newly discovered SNPs. SNPs were genotyped using technologies developed by Perlegen, Illumina, Third Wave, Sequenom, ParAllele, and Perkin-Elmer. Four quality assessment exercises found that the data are > 99% complete and > 99.5% accurate. The HapMap data are freely and publicly available at the HapMap Data Coordination Center (www.hapmap.org and hapmap.jst.go.jp) and dbSNP (www.ncbi.nlm.nih.gov/SNP), and are incorporated into the UCSC Genome Browser and Ensembl. The DNA samples were collected through processes of community engagement and public consultation with individual informed consent. The 270 samples included 30 Yoruba trios from Ibadan, Nigeria, 45 Japanese from Tokyo, Japan, 45 Han Chinese from Beijing, China, and 30 Utah CEPH trios (northern and western European ancestry). Analyses of the data were performed on both the ENCODE genotypes and the genome-wide HapMap genotypes to assess how sensitive the inferences of patterns of LD and choice of markers for tagging haplotypes are to SNP density. Specifically, we analyzed the types and patterns of LD and common haplotypes, estimated a fine-structure recombination map, searched for associations with genomic features, and sought evidence for natural selection as an explanation of specific patterns. All analyses were conducted genome-wide, for each population sampled.

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Effect of mutation type and location on clinical outcome in 1081 Marfan syndrome patients or related phenotypes with FBN1 mutations : an international study. *L. Faivre[1], G. Beroud[2], A. Child[3], B. Callewaert[4], C. Binquet[1], E. Gautier[1], E. Arbustini[5], A. Kiotsekoglou[3], P. Comeglio[3], C. Beroud[2], C. Bonithon[1], D. Halliday[6], C. Muti[7], L. Ades[8], J. De Baecker[4], P. Coucke[4], U. Francke[9], A. De Paepe[4], G. Jondeau[7], C. Boileau[7].* 1) Centre dépidémiologie - investigation clinique et centre de Génétique, CHU Dijon, France; 2) IURC, Montpellier, France; 3) St. Georges Hospital, London, UK; 4) Ghent University Hospital, Belgium; 5) Policlinico San Matteo, Pavia, Italy; 6) University of Oxford, UK; 7) Hôpital Ambroise Paré, Boulogne, France; 8) Royal Alexandra Hospital, Sydney, Australia; 9) Stanford University Medical Center, USA.

Mutations in the FBN1 gene cause Marfan syndrome (MFS) and have been associated with a wide range of milder overlapping phenotypes. A large collaborative study based on the international FBN1 mutation database including 1081 patients (820 probands) allowed us to investigate the relationship between the FBN1 genotype and the nature and severity of the clinical phenotype. A set of qualitative and quantitative clinical parameters was compared for different classes of mutation. Patients with a FBN1 null mutation had a more severe skeletal, aortic and lung phenotype than patients with a mutation producing an altered protein (p=0.005, p=0.0004 and p=0.007), whereas the frequency of ectopia lentis was lower (p0.0001). A higher risk of ectopia lentis was found in patients with a missense mutation substituting or producing a cysteine when compared to patients with other missense mutations (p0.0001), as well as with a mutation within the 5 region versus 3 (p=0.0003). Patients with mutations within exons 24-32 had a more severe phenotype (younger age at diagnosis, at aortic dilatation or aortic surgery and shorter survival) than patients with mutations located elsewhere (p0.0001), even when neonatal MFS were excluded. No significant differences were found for any clinical parameter in patients with a mutation located in EGF domains versus TGFBP domains. This study is the largest ever reported and indicates high-risk groups for cardiac and ophthalmologic manifestations, and can be helpful in monitoring patients with a FBN1 mutation.

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A meta-analysis of FXTAS patients with and without family history of fragile X syndrome: a probable threshold model for the toxicity of CGG repeats. *S. Jacquemont[1], L. Beckett[2], M. Leehey[3], F. Tassone[4], R. Hagerman[5], P. Hagerman[3].* 1) Département de Génétique, C H U, Nantes, France; 2) Division of Biostatistics, Department of Public Health Sciences, UC Davis, CA; 3) Department of Neurology, University of Colorado Health Sciences Center Denver, CO; 4) Department of Biochemistry and Molecular Medicine, School of Medicine, University of California Davis, CA; 5) MIND Institute, University of California Davis Medical Center Sacramento, CA.

Results of genetic screening for the FMR1 premutation (55-200 CGG repeats) have been published by 12 independent groups. 2820 patients were ascertained through movement disorder clinics independent of their family history. We conducted a meta analysis in order to determine the prevalence of FXTAS for the main movement disorder diagnoses and established guidelines for fragile X premutation screening in neurology clinics. The overall prevalence of the FMR1 premutation regardless of the movement disorder diagnosis is 1%, 7 times greater than expected based on the prevalence of the premutation in males of the general population (OR = 6.9 ; 95% CI : 1.8 , 65.5). This figure has limited relevance since the excess of premutation allele was significant only in the group of males presenting cerebellar ataxia after the age of 50 years. In that group, the prevalence of the premutation rises to 2.7% ; (21/778), 22 times greater than expected based on the prevalence of the premutation in males of the general population (OR = 22.4 ; 95% CI : 5.8, 86.7). The clinical descriptions of these patients were compared to the cases identified through fragile X families. The meta analysis of the molecular data from patients recruited with or without a family history of fragile X supports the hypothesis of a threshold model of the toxicity of CGG repeats. We conclude that the toxicity of CGG repeats below 70 may not have functional repercussions or may be associated with FXTAS related disorders. This meta-analysis has consequences for the projected prevalence of FXTAS in the general population, which is lower than the initial estimate of 1/ 3000.

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FXTAS: a descriptive study of premutation carriers from fragile X families. *E.G. Allen, J. Juncos, M. Rusin, G. Novak, L. Shubeck, S.L. Sherman, R. Letz.* Emory Univ, Atlanta, GA.

We are conducting a study to further examine the symptoms, penetrance, and risk factors associated with the tremor/ataxia syndrome (FXTAS) among carriers of premutation alleles of the FMR1 gene. Our study population includes all siblings of premutation carrier males over the age of 50 identified through a survey of families with fragile X syndrome. We conducted a comprehensive battery of tests including a medical history, a neuropsychological test battery, and quantitative neurological assessment. Within the neurological assessment, we use a series of tests to obtain objective, quantitative measures of key features observed in FXTAS cases to date: 1) resting, postural, and intention upper-limb tremor, 2) reduced vibration sensation, a surrogate of possible neuropathy, 3) decreased postural stability, and 4) dysdiadokinesis, or upper limb uncoordination. We have obtained these measures on 49 males (mean age=63.8) and 20 females (mean age=65.3). Subjects were scored as expressing the above phenotypes if they scored above the 95th percentile of age-adjusted standards. Among premutation males, we have seen a significantly increased incidence of tremor (45%), peripheral neuropathy (14%), decreased postural stability (57%), and dysdiadokinesis (78%). In contrast, these phenotypes were not markedly increased in females or non-carrier male relatives. These findings as well as neuropsychological measures will be examined with molecular correlates of FMR1.

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Spectrum of *CHD7* mutations in 113 patients with CHARGE Syndrome. *S.R. Lalani[1], A.M. Safiullah[1], S.D. Fernbach[1], L.M. Molinari[1], C. Bacino[1], S.L. Davenport[4], M.A. Hefner[2], J.M. Graham Jr.[3], J.W. Belmont[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, St. Louis University School of Medicine St. Louis, MO; 3) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 4) Sensory Genetics/Neuro-Development, St. Paul, Minnesota.

CHARGE is a complex birth defect, characterized by non-random occurrence of coloboma, choanal atresia, cranial nerve defects, distinctive inner ear abnormalities, heart and urogenital anomalies and growth retardation. Recently, intragenic mutation of *CHD7*, the chromodomain-helicase DNA binding protein was reported to be a major cause of CHARGE syndrome. Chromatin remodeling is a well-recognized mechanism of gene expression regulation and the gene is likely to play a significant role in embryonic development and cell cycle regulation. We report the spectrum of *CHD7* mutations in 113 individuals with sporadic and familial CHARGE. Mutations were found in 62 patients (55%) distributed throughout the gene. About 68% were truncating mutations, most likely leading to haploinsufficiency. We have obtained phenotypic information on all patients and have performed multivariate analysis conditioned on the mutation status. The analysis shows that congenital heart defects (50/55 compared to mutation negative group, p=0.014) and growth retardation (42/48 compared to mutation negative group, p=0.002) are more frequent in patients with *CHD7* mutation. Mouse embryo in situ hybridization shows expression of this gene in the oto-acoustic complex, brain, ear, pharyngeal endoderm, and heart tube. We have also performed microarray analysis of gene expression using lymphoblastoid cell lines of seven *CHD7* mutation positive patients and compared the expression pattern in these to five control subjects and four affected patients with no abnormality of *CHD7* gene by sequence analysis. Preliminary analysis shows significant gene expression differences among these three groups. The differential gene expression pattern in the *CHD7* mutation negative group may further assist in understanding the molecular basis of disorder in this group of affected children.

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Phenotypic spectrum of CHARGE syndrome with CHD7 mutations. *K. Kosaki[1], M. Aramaki[1], T. Udaka[1], R. Koaki[2], Y. Makita[3], N. Okamoto[4], H. Yoshihashi[5], H. Oki[5], Y. Fukushima[6], H. Kawame[7].* 1) Dept Pediatrics, Div Med Gen, Keio Univ, Tokyo, Japan; 2) Dept of Clin Genet & Mol Med, Nat'l Children'fs Med Ctr, Tokyo, Japan; 3) Dept of Pediatrics, Asahikawa Med Col, Asahikawa, Japan; 4) Department of Planning and Research, Osaka Medical Center and Res Inst for Maternal and Child Health, Osaka, Japan; 5) Tokyo Metropolitan Kiyose Children'fs Hosp,Tokyo; 6) Dept of Med Genet, Shinshu Univ Sch of Med, Matsumoto, Japan; 7) Division of Clinical Genetics, Nagano Children's Hosp, Nagano, Japan.

CHARGE syndrome is characterized by a constellation of non-randomly associated malformations: C - coloboma of the iris or retina, H - heart defects, A - atresia of the choanae, R - retardation of growth and/or development, G - genital anomalies, and E - ear abnormalities. Recently, Vissers et al. identified the gene Chromodomain helicase DNA-binding protein-7 (CHD7) at chromosome 8q12.1 as a causative gene of CHARGE syndrome. We further delineated the phenotypic spectrum of CHARGE patients with mutations in CHD7. Twenty-three patients who fulfilled Blake'fs criteria were screened for CHD7 mutations by using DHPLC. PCR products corresponding to all variant elution profiles were sequenced bidirectionally using the dideoxy sequencing method. We identified heterozygous CHD7 mutations in 17 (71%) of the 24 patients enrolled in the study: 7 frameshift mutations, 6 nonsense mutations, 3 splice-site mutations, and 1 intragenic deletion from exon 8 to 12. These mutation classes are likely to result in a prematurely truncated protein or the loss of protein expression. Altogether, 15 of the 17 cases had coloboma of the eyes. However, only 2 patients had iridal coloboma and 5 patients had only disc coloboma, without iridal or retinal coloboma. Thus, the retina and optic disc must be thoroughly examined when CHARGE syndrome is suspected, even in the absence of iridal coloboma. Choanal atresia/stenosis was not common (5 patients) and was less frequent than oral clefts (8 patients). Severe hearing-loss, laryngomalacia, and developmental delays were prevalent. Genetic testing of CHD7 will be helpful in confirming the diagnosis and in providing accurate genetic counseling to the families.

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Genotype-phenotype correlations in Rubinstein-Taybi syndrome. *E.K. Schorry[1], M. Keddache[1], B. Abiramikumar[1], N. Lanphear[2], J. Rubinstein[2], G.A. Grabowski[1].* 1) Div. of Human Genetics; 2) Div. of Developmental Disabilities, Cincinnati Children's Hospital.

Rubinstein-Taybi syndrome (RTS) is a well-described multiple congenital anomaly/mental retardation syndrome which usually occurs sporadically. Loss of function in CREBBP or EP300 genes, which encode histone acetyl transferases, has been found in about 50% of patients with RTS, with the majority of mutations in CREBBP. We performed mutation analysis of CREBBP in 98 patients who met diagnostic criteria for RTS during 2 international RTS family conferences. DNA was extracted from peripheral blood, and mutation analysis of CREBBP was performed on all 31 coding exons and exon-intron junctions by bidirectional comparative PCR sequencing. A total of 59 different variations were observed in the DNA sequence. All mutations involving a change in the protein were unique, and were equally distributed throughout the 31 coding exons. Twenty-five of the mutations created a truncated protein product or clearly altered the splice-donor/splice-acceptor consensus sequence; 11 mutations resulted in single amino acid changes. Forty-five patients did not have an identifiable mutation in CREBBP.

Extensive phenotypic data was also collected on the patients during the conference. All patients had the characteristic facies, broad thumbs, and mental retardation. We analyzed phenotypic data to determine correlations with specific mutation types, i.e., truncating, splice site, single amino acid substitutions, or no identifiable mutation. There were no differences in the facial and broad thumb phenotype between the 4 groups, with all groups displaying the characteristic dysmorphology. Degree of mental retardation and growth retardation were similar in all groups. Congenital heart disease was seen in 40% of the group overall, but was less frequent (18%) in patients with single amino acid substitutions. Interestingly, duplication of the great toe was seen in 8 patients with no identifiable mutation, but in none of the patients with CREBBP mutations. Further research is needed to determine the additional genes which cause this phenotype.

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Phenotypic characterization of familial oculo-auriculo-vertebral (OAV) spectrum: assessment of 19 additional families. *A.E. Beck[1], L. Hudgins[2], A.W. Grix[4], N.H. Robin[5], E. Chen[6], L.C. Lazzeroni[3], H.E. Hoyme[2], U. Francke[1,2].* 1) Dept Genetics and; 2) Dept Pediatrics, Div Medical Genetics and; 3) Dept Health Research & Policy, Div Biostatistics, Stanford Univ, Stanford, CA; 4) Permanente Medical Group, Sacramento, CA; 5) Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL; 6) Permanente Medical Group, Oakland, CA.

Although the OAV spectrum is considered a sporadic condition, a few large studies suggest that up to 45% of probands with hemifacial microsomia or microtia have affected relatives. In the present study, we evaluated 19 families in which probands with OAV had additional family members with OAV features. We ascertained 54 affected family members (including probands) and 18 normal relatives who were apparently transmitting carriers. Of those phenotypically affected, 57% were female and 43% were male. 63% had unilateral and 37% had bilateral findings. 93% exhibited auricular anomalies including preauricular tags (23 cases) and microtia (30 cases, often with canal narrowing/atresia and conductive deafness). Eye malformations in 20% included epibulbar dermoids (8/11), microphthalmia, and iris/retinal colobomas. Mild facial asymmetry was seen in 30%, but the marked hemifacial microsomia often seen in sporadic OAV was uncommon. Renal (4%), vertebral (11%) and cardiac (7 %) anomalies occurred less frequently than in sporadic cases. Only 2 affected subjects had intellectual impairment. Chromosomal studies in probands were normal. Although mouse models and candidate regions in the human genome are under investigation, no human gene mutations for OAV are known. Our new data suggest that there are genetic subtypes within the OAV spectrum consistent with autosomal dominant and possibly X-linked inheritance of a single gene displaying variable expressivity and incomplete penetrance. Careful evaluation of relatives for minor manifestations of OAV is essential before quoting recurrence risks. Since microtia can result from aberrant migration of neural crest cells into the 1st/2nd branchial arches during embryonic weeks 4-5, it is tempting to postulate that the altered gene(s) in our familial cases are involved with neural crest regulation.

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Array CGH identifies chromosome abnormalities with unexpected clinical variability in contiguous gene syndromes. *B.A. Bejjani, B.C. Ballif, C.D. Kashork, E. Rorem, K. Sundin, L.G. Shaffer.* Signature Genomic Laboratories, LLC, Spokane, WA.

Array CGH provides distinct advantages over conventional cytogenetics by detecting aneuploidy, microdeletions, microduplications, and subtelomeric rearrangements in a single, simultaneous assay. The SignatureChip was designed to detect the common microdeletions, reciprocal microduplications, subtelomeric and pericentromeric alterations, unbalanced translocations, and aneuploidy, while avoiding most common population variants. The design also allows for distinguishing the common-sized microdeletions that are flanked by low copy repeats from larger deletions through the use of flanking control loci. The array uses 831 BACs covering 126 clinical and 104 control loci in 3-6 clone contigs. Of the 1,300 clinical cases submitted to our laboratory for diagnostic testing, we identified 73 cases (5.6%) with clinically relevant abnormalities. The majority of these cases have had at least one previous cytogenetic study. Among these, 22 telomeric deletions or unbalanced rearrangements and 21 cases with syndromic deletions were identified. We also identified 3 cases of interstitial deletions of 1p36 and three cases of interstitial duplications on 3q, 4q and 16q, none of which would be detected using available sets of subtelomere probes. The finding of 21 cases with syndromic microdeletions most of which were submitted by clinical geneticists after at least one previous cytogenetic study suggests that these well-characterized syndromes have unrecognized variability in clinical presentation that may prevent the clinician from readily reaching a diagnosis. The clinical variability of these syndromes will be reviewed. In addition, microduplications of 15q12, 17p11.2, and 22q11.2 have been identified, confirming that reciprocal products of common microdeletion syndromes will be detected. These microduplications are unlikely to be identified by conventional metaphase FISH assays. Thus, array CGH is a powerful approach for uncovering subtelomeric rearrangements, microdeletions and microduplications, even in patients who are not suggestive of a particular syndrome.

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9q34.3 microdeletion syndrome: clinical and genetic insights. *S.A. Yatsenko[1], H. Firth[4], S. Tomkins[5], O. Rittinger[6], E. Lammer[7], K.S. Lewis[8], S.W. Cheung[1], P. Stankiewicz[1], J.R. Lupski[1,2,3].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine; 3) Texas Children Hospital, Houston, TX; 4) Addenbrookes Hospital, Cambridge, UK; 5) St. Michael's Hospital, Bristol, UK; 6) Klinische Genetik, Landeskinderklinik Salzburg, Salzburg, Austria; 7) Children's Hospital and Research Center at Oakland, Oakland, CA; 8) St. Joseph's Hospital And Medical Center, Phoenix, AZ.

9q34.3 microdeletion syndrome is a contiguous gene syndrome characterized by craniofacial dysmorphism, neonatal hypotonia, childhood obesity, microcephaly, mental retardation and absence of expressive speech. Depending on the deletion size, additional clinical features can include congenital heart defects, seizures, abnormal male genitalia, limb and brain anomalies. Recently we identified an ~700 kb critical region at the most distal portion of 9q34.3 encompassing genes *EHMT1* (euchromatic histone methyltransferase) and *CACNA1B* in association with a minimal phenotype (Yatsenko et al. 2005). Haploinsufficiency of *EHMT1* in all our patients and disruption of *EHMT1* identified in a child with features of 9q deletion syndrome (Kleefstra et al. 2005) provide the first evidence that dysregulation of histone modifications and chromatin structure can be a pathophysiological mechanism underlying mental retardation and neurobehavioral disorders.

Here we present the FISH mapping of the breakpoints in six new patients with del(9)(q34.3) using BAC and fosmid clones, and compare the deletion sizes and clinical manifestations. We constructed a deletion map of 9q34.3 chromosome region using cell lines from eleven affected individuals. In addition molecular cytogenetic analysis revealed one patient with der(9)del(9)(q34.3)dup(9)(q34.3q34.2). Our investigation showed that the 9q34.3 deletions varied from ~700 kb to 3.5 Mb in size and complexity of the rearrangement can be detected only by FISH or microarray CGH analyses. These observations provide new insights for genetic counseling and for the search of new candidate genes. We discuss genotype-phenotype correlation, and the possible molecular mechanism of deletion.

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Overgrowth in association with chromosomal anomalies. *G.S. Ball, R.K. Aldrich, J. Lee, S. Li, K.A. Casas.*
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Overgrowth is a feature of very few chromosomal syndromes. We report two new patients with overgrowth. Patient 1 presented at birth with distal joint contractures and failed newborn hearing screen. Imaging studies revealed agenesis of the corpus callosum and left cystic kidney. Routine chromosome analysis performed shortly after birth showed extra material on chromosome 6q. Subtelomeric fluorescent in situ hybridization (FISH) using a 10p subtelomeric probe confirmed that the extra material on 6q was derived from 10p, resulting in terminal deletion of 6q and trisomy 10p. At 5 months of age, her length was 4 standard deviations above the mean for age. Weight and occipitofrontal circumference were at the 75th centile. Palm length was greater than the 97th centile for age, and there was an advanced bone age of 2 years. Patient 2 presented at 15 years of age with mild mental retardation and recent onset of seizures and hypertension. Her height was over 3 standard deviations above the mean for an adult female, weight was 4 standard deviations above the mean for an adult female, and occipitofrontal circumference was 3 standard deviations above the mean for an adult female. Palm length was at the 97th centile for an adult female. Routine chromosome analysis had been performed in early childhood with normal results. Subtelomeric FISH testing at 15 years of age revealed an unbalanced rearrangement with deletion of 8p and trisomy of 12p. Overgrowth is not a reported feature of the 6q deletion or 10p duplication syndromes, suggesting a fusion gene or other mechanism unique to the (6;10) translocation in Patient 1. Overgrowth is not a reported feature of the 8p deletion syndrome, but increased birth length and birth weight have previously been associated with duplication 12p and tetrasomy 12p, suggesting a dosage effect of a growth factor gene. Results in Patient 2 narrow the candidate region for this gene(s) to the 12p subtelomere.

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Identification of Segmental Chromosomal Duplications and Deletions in Prostate Cancer. *A. Pearlman, M. Salman, H. Ostrer.* Human Genetics Program, New York University School of Medicine, New York, NY.

Recurrent somatic genetic alterations contribute to the development and progression of prostate cancer. These events can be identified using arrayCGH, a high-resolution technique that maps the duplicated or deleted segments onto arrays of well-characterized bacterial artificial chromosomes (BACs) or oligonucleotides. As part of our efforts to identify somatic alterations that are predictive of racial differences or prognosis, and to aid the identification of tumor suppressor genes, we compared the performance of Affymetrix 10K SNP chips and 19K BAC arrays using three Gleason 7 prostate cancers and their matched normal tissue. We analyzed the Affymetrix 10K SNP chip arrays using GDAS 2.0, dChipSNP and the Affymetrix copy number tool and the BAC arrays using the CGH Explorer, DNAcopy, vMAP, and GLAD segmentation tools. SNP chips provided calls for 93-98% of the probes. The gender calls were as expected for all samples and the concordance between replicates was 97%. No duplication events were identified with confidence. LOH events were observed on chromosomes 1, 13, and 21 for tumor 3 and in an overlapping region on 13 for tumor 1. Among the 60,000 events sampled, 4 small, LOH events occurred in single samples, but not in their replicates, suggesting that the frequency of false positives was <10-4. BAC arrays demonstrated duplications and deletions on multiple chromosomes for each sample, some of which overlapped. Applying CGH Explorer segmentation to the recurrent chromosome 13 deletion, we observed identical proximal breakpoints for tumors 2 and 3 and an overlapping breakpoint for tumor 1. Different distal breakpoints were observed for all 3 tumors. Sensitivity analysis using this program did not alter the segmentation on chromosome 13, nor create new chromosome 13 segments for the corresponding normal samples, indicating the robustness of the data and the tool. We compared the different methods for precision of segmentation and observed virtually identical breakpoints. These observations demonstrate that BAC arrays and SNP chip arrays provide precise and complementary identification of different types of chromosomal events in prostate cancer.

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Finding prostate cancer susceptibility genes using linkage-based and candidate gene approaches. *E.A. Ostrander, on behalf of The International Consortium of Prostate Cancer Genetics.* Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD.

Identification of prostate cancer (PC) susceptibility genes has been hampered by the phenotypic heterogeneity associated with the disease, the large number of contributing loci, and the associated difficulties in accurately stratifying families based on features of disease, family history, or clinical presentation. Although genome-wide screens have been performed in over a dozen independent studies, few chromosomal regions have been consistently identified as regions of interest. The International Consortium for Prostate Cancer Genetics (ICPCG) was formed to facilitate the identification of PC susceptibility genes. The ICPCG has undertaken analyses aimed at utilizing the large family resources available (over 1800 PC families). These include: 1) combining genome-wide screen linkage data from a total of 1,233 PC families collected by members of the ICPCG. Using parametric and non parametric analyses five regions were identified with suggestive linkage (LOD score >1.86) on 5q12, 8p21, 15q11, 17q21 and 22q12, as well as a significant linkage at 22q12 (LOD 3.57) in a subset of families with 5 or more affected members. These findings are consistent with the hypothesis of multiple PC susceptibility genes with modest effects, or several major genes segregating in small subsets of families; 2) multi-locus analysis of genome wide scan data in all families using ordered subset analysis and pedigree covariate analysis using recursive partitioning; 3) a genome wide scan for linkage in 189 families that have three or more first degree relatives with aggressive prostate cancer; 4) genome wide scans for linkage in families with both prostate and other cancers (e.g. gastric cancer) and prostate cancer families of Ashkenazi Jewish descent. Finally, we have undertaken replication studies for previously described loci on the X chromosome, and testing of provocative candidate genes such as CHEK2 and NBS1. In addition, novel methodologic approaches are being developed and implemented to analyze this large and informative data set.

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Genome-wide linkage scan for prostate cancer susceptibility loci in men with aggressive disease: The University of Michigan Prostate Cancer Genetics Project. *L.A. Ho[1], J.B. Dimmer[2], Y. Wang[1], E.M. Gillanders[3], K.A. Cooney[2], E.M. Lange[1].* 1) University of North Carolina, Chapel Hill, NC; 2) The University of Michigan, Ann Arbor, MI; 3) NHGRI, NIH, Bethesda, MD.

Identifying prostate cancer susceptibility genes using linkage analysis has proven to be a difficult challenge. It is clear that there is considerable genetic heterogeneity which results in reduced power to detect linkage signals. Another likely complication in mapping prostate cancer susceptibility genes is that there is considerable heterogeneity in the phenotype of prostate cancer, with a considerable range in the aggressiveness of the disease even among family members. We performed a genome-wide nonparametric linkage scan for genes that predispose to aggressive forms of the disease based on 405 genetic markers and 79 informative pedigrees with two or more cases of aggressive disease. Only individuals defined to have aggressive disease were coded as "affected" in our analyses. An indicator variable for aggressive disease was created using the following criteria: 1) Regional or distant stage (based on pathology if radical prostatectomy done, otherwise clinical stage, T3, T4, N1, M1) or 2) Gleason score of 7 or higher or poorly differentiated grade (if no Gleason score is available) or 3) PSA at diagnosis 20 or higher or 4) prostate cancer listed as primary cause of death on death certificate. We found strong evidence for linkage on chromosome 15q (LOD = 3.43) near marker D15S1002. Suggestive linkage was found on chromosome 6p (LOD = 2.21) near marker D6S422. Additional LOD scores greater than 1 were found on chromosomes 3q (1.43), 18q (1.17), 20q (1.05) and Xp (1.02). Using a more rigid definition of prostate cancer will result in a severe reduction in the effective sample sizes available for linkage analysis. However, the reduction in heterogeneity or "noise" in the phenotype may ultimately prove to increase the statistical power to detect susceptibility genes for this complex trait.

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Single locus gene mutations and prostate cancer: Can mutations in the androgen receptor gene be directly linked to the occurrence of prostate cancer? *B. Gottlieb[1,3,5], C. Alvarado[1], L.K. Beitel[1,2], K. Sircar[4], A. Aprikian[4], M. Trifiro[1,2,3].* 1) Dept Cell Genetics, Lady Davis Inst Medical Res, Montreal, PQ, Canada; 2) Dept Medicine, McGill University, Montreal, PQ, Canada; 3) Dept Human Genetics, McGill University, Montreal, PQ, Canada; 4) Dept Urology, McGill University, Montreal, PQ, Canada; 5) Dept Biology. John Abbott College, Ste Anne De Bellvue, PQ, Canada.

One of the Holy Grails of cancer genetics has been to find mutations in a specific gene linked directly to a specific cancer. A possible candidate has been the androgen receptor gene (*AR*), which recent evidence has shown is important in all prostate cancer (CaP) stages. However, numerous studies that have examined the possible association between *AR* mutations and CaP have produced very inconclusive results. To resolve this issue we have considered the fact that tumor tissue heterogeneity has rarely been considered an indicator of genetic heterogeneity. To see if *AR* genetic heterogeneity does indeed exist we have conducted a micro-genetic examination of tumors from CaP patients using laser capture microdissection (LCM) to isolate specific pathologically-identified tissues and then sequence their *AR*. To help correlate mutations with tissue type, we have prepared cancerous and benign tissue micro-arrays from prostates from men with CaP. To date we have found a number of different mutations, in distinct areas isolated from a single prostate, all of which appear to be unique. Mutations were found in both cancerous and benign tissue from the central zone of the prostate, but not from benign peripheral and transitional zones. Even more intriguing is that different mutations were found in cancerous and benign tissues. Thus, it appears that somatic mosaicism of the *AR* occurs within CaP tumors, although the exact relationship between *AR* mutations and CaP must await an analysis of many more prostate tumors. We believe that this study, which is the first to conduct a micro-genetic analysis of *AR* mutations in CaP by utilizing LCM, points the way to an approach that can much more accurately monitor genetic events that lead to the initiation and progression of cancer.

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Allele-specific expression in familial pancreatic cancer. *J. Fan[1], A. Maitra[2], M. Bibikova[1], E. Chudin[1], E.W. Garcia[1], D. Barker[1], P. Chen[2], C. Karikari[2], M.G. Goggins[2], R.H. Hruban[2], A. Chakravarti[2].* 1) Illumina, Inc, San Diego, CA; 2) Johns Hopkins University, Baltimore, MD.

Pancreatic cancer afflicts over 30,000 individuals each year in the United States, and is mostly lethal within months of diagnosis. About 10% of pancreatic cancer is familial due to an inherited predisposition. Despite considerable effort, germline mutations have been identified in less than 20% of families, in a few genes such as BRCA1/2. To enhance gene discovery, we analyzed germline allele-specific expression (ASE) patterns in familial pancreatic cancer (FPC). Our central hypothesis is that the gene responsible for FPC would be hemizygously mutated in the germline of affected individuals, and hence, would only or predominantly be expressed hemi-allelically from the wild-type allele (at least for a subset of mutations such as nonsense mutations or deletions). A microarray-based ASE assay was developed for 2,140 coding SNPs (cSNPs) derived from 680 cancer-associated genes, such as cell cycle checkpoint and apoptosis-inducing genes. Lymphoblastoid cell lines from 48 age-matched individuals (16 CEPH family trios) were analyzed for genomic heterozygosity and allele-specific differences in expression, if any, at heterozygous cSNPs. Genes demonstrating significant ASE in these normal individuals were catalogued for reference. Subsequently, lymphoblastoid cell lines from 109 individuals with FPC were examined for ASE variations. Overall, 331 genes demonstrated potential ASE in at least one of the 157 samples, of which 201 demonstrated ASE in both CEPH and FPC samples. These include known imprinted genes as well as multiple X-chromosome transcripts, confirming the overall validity of our platform. The remaining 130 genes demonstrated ASE in one or more of the 109 FPC patient samples only, and include both known genes implicated in FPC (e.g., BRCA1/2) as well as novel candidate genes (e.g., XRCC5, BPAG1, AGO, etc.). We are currently validating array-based ASE on a gene-by-gene basis using quantitative RT-PCR. Mutational screening will be performed for genes that display confirmed ASE variation in FPC patients but not (or at a significantly lower frequency) in CEPH controls.

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***PRKAR1A* mutations leading to Carney complex, PPNAD and related disorders: Analysis of the largest database to date, identification of large gene rearrangments and other novel disease-causing alterations, and functional characterization of expressed mutants.** *A. Horvath, S. Boikos, F. Weinberg, E. Meoli, S. Stergiopoulos, T. Bei, L. Matyakhina, I. Bossis, C. Stratakis.* Section on Endocrinology & Genetics, DEB, NICHD, NIH, Bethesda, MD.

Carney complex (CNC) and primary pigmented nodular adrenocortical disease (PPNAD) are caused by inactivating mutations in *PRKAR1A*, the main regulatory subunit of cAMP-dependent protein kinase (PKA). We have screened for *PRKAR1A* sequence changes a total of 655 individuals from 171 non-related affacted kindreds, by DHPLC and genomic sequencing; negative samples were then screened by Southern blotting; 161 patients have been found to have 55 (33 novel) different disease-causing single base substitutions or small insertions/deletions scattered over the coding sequence and the splice junctions. Six index cases have shown alternative patterns in heterozygote state by Southern analysis using EcoRI; further analysis confirmed two distinct deletions, one ~5 kb from the exon3/intron3 region, and another deleting ~4 kb from between exons 4 and 8. These data were confirmed by long distance PCR; FISH did not show any abnormalities. Four specimens were sharing common EcoRI altered restriction pattern, suggesting mutations of EcoRI site. In contrast to previous studies, where the vast majority of the mutations were proven to lead to NMD, and subsequently, not to be expressed, we identified 10 different expressed mutations, which allowed us to assess the genotype-phenotype correlations. Two of the expressed mutations were associated with particularly aggressive clinical phenotype, two other were associated almost exclusively with PPNAD only. We conclude that (1) there is a large number of new PRKAR1A mutations associated with CNC and PPNAD, (2) the existence of large gene rearrangements confirms the complex *PRKAR1A* disease-mutation spectrum (3) approximately 20% of the mutations do not undergo NMD and lead to the expression of various mutant PRKAR1A proteins, and (4) there is genotype-phenotype correlation. This study has important implications for counseling, and for the molecular pathophysiology of PKA-associated tumorigenesis.

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A mouse model employing the tet-off system in an antisense construct to *Prkar1a* confirms tTA-independent expression of the transgene and develops tumors consistent with Carney complex (MIM160980). *K.J. Griffin, F. Weinberg, S. Stergiopoulos, L. Matyakhina, L. Kirschner, C. Stratakis.* SEGEN, DEB, NICHD,NIH, Bethesda, MD.

Carney complex (CNC) is a multiple neoplasia syndrome associated with adrenal, pituitary, thyroid, and gonadal neoplasms, caused by *PRKAR1A* mutations inactivating the type IA regulatory subunit of protein kinase A (PKA). A transgenic line carrying an antisense transgene for exon 2 (X2as) of *Prkar1a* was crossed with a tTA-transgene-expressing line. The resulting tTA/X2as mouse mimicked CNC in many respects (Cancer Res 2004;64:8811, J Med Genet 2004;41:923), and developed lymphoid hyperplasia. The mice with only the X2as transgene also developed abnormalities that were not present in mice carrying tTA alone or in wild type mice of the same background. Previous reports have suggested that the tet-off system is leaky, allowing for the occasional expression of the target construct without the tTA factor. This study reports 136 mice: wild type (16), those carrying only the X2as transgene (60), those expressing tTA only (15), and the tTA/X2as (45) line published recently. The X2as mice developed a variety of tumors that were not present in controls, and not different from those in tTA/X2as mice. These include follicular adenomas of the thyroid, pigmentation and x-zone vacuolization of the adrenal cortex, uterine cysts, and abnormalities of the testis. The X2as mice did not develop lymphoid hyperplasia or lymphoma as frequently as tTA/X2as mice. PKA activities in X2as tissues were intermediate between tTA/X2as and controls. Genetic analysis of X2as-derived sarcomas showed involvement of the mouse chromosome 11 *Prkar1a locus*. Expression of the X2as transgene was confirmed by protein and mRNA studies; proliferation assays of X2as and tTA/X2as mouse-embryonic fibroblasts were similar. We conclude that the X2as mice developed tumors similar to those seen in CNC, tTA/X2as, and $Prkar1a^{+/-}$ mice. These studies confirm the leakiness of the tet-off system but also provide the opportunity to test which features are related to *Prkar1a*-down regulation vs. expression of the tTA transgene.

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Mutations and Interstitial Deletions Involving Exon 3 of the Beta-catenin Gene are Detected in Sporadic Adrenocortical Tumors. *I. Bourdeau, A. Lampron, M. Tadjine.* Division of Endocrinology, Department of Medicine, Centre hospitalier de lUniversité de Montréal (CHUM)-Hôtel-Dieu, Montréal, Québec, Canada H2W 1T8.

Adrenocortical lesions are diagnosed frequently as benign adenomas or nodular hyperplasias, and more rarely as malignant adrenocortical carcinomas. The genetic background of sporadic adrenocortical lesions is poorly characterized. In our previous work, involving large-scale cDNA microarray analysis, we demonstrated aberrant differential expression of a number of Wnt/-catenin signaling-related genes implicated in adrenocortical hyperplasias. To further explore the role of Wnt/-catenin signaling in adrenocortical tumorigenesis, we conducted a search for mutations of exon 3 of the -catenin gene. DNA was extracted from 48 human adrenocortical samples and the human adrenocortical cancer cell line NCI-H295R. The adrenal tissue samples included 28 patients with adenomas, 4 with carcinomas, 13 with ACTH-independent macronodular adrenal hyperplasia (AIMAH) and 3 with ACTH-dependent adrenal hyperplasia. The samples were screened for somatic mutations in exon 3 of the -catenin gene using deletion screening by polymerase chain reaction (PCR) and direct sequencing. We found genetic alterations in 6 out of 28 adenomas (21,4%). There were no mutations detected in adrenocortical carcinomas, AIMAH, ACTH-dependent hyperplasia and the NCI-H295R cell line. Three point mutations occurred at potential serine/threonine phosphorylation residues of codons 37 or 45 [S37C (n=2) and S45F n=1)]. The remaining 3 tumors contained deletions of 6, 55 and 271 bp, each including part of exon 3. Reverse transcription-PCR experiments with RNAs isolated from the adenoma with the 271bp deletion detected transcripts that lacked exon 3, in addition to the normal transcript. These mutations, as previously reported in several types of tumors, but never in adrenocortical tumors, abrogate the phosphorylation-dependent interaction of -catenin known to lead to transcription activation of Wnt target genes. Our results suggest, for the first time, that -catenin gene mutations are frequent in adrenocortical adenomas. Possible involvement of -catenin activation could contribute to adrenocortical tumorigenesis.

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Genome-wide localization of menin and assocated histone methytransferase complex members. *P.C. Scacheri[1], S. Davis[1], D.T. Odom[2], S. Perkins[1], A.M. Spiegel[3], P.S. Meltzer[1], F.S. Collins[1].* 1) NHGRI, Bethesda, MD; 2) Whitehead Institute, Cambridge, MA; 3) NIDCD, Bethesda, MD.

Multiple Endocrine Neoplasia, type I (MEN1) is a familial cancer syndrome characterized primarily by multiple tumors of the endocrine glands. The gene for MEN1 encodes a ubiquitously expressed tumor suppressor protein called menin. Menin was recently shown to interact with several components of a trithorax family histone methyltransferase complex including ASH2, Rbbp5, WDR5, and the leukemia proto-oncoprotein MLL. These findings suggested a role for menin in transcriptional regulation, potentially mediated through covalent modification of histone H3 at lysine 4. To gain insights into menin's role as a tumor suppressor, we determined the genomic occupancy of menin, MLL, and Rbbp5, using a strategy that couples chromatin immunoprecipitation with DNA microarray technology (ChIP-chip). The microarrays used in this approach were custom designed to contain high density oligonucleotide tiling paths across 20,000 human promoter segments. Given that MLL is reported to regulate homeobox (HOX) transcription, these arrays also harbored tiling paths across all four HOX clusters. Our data in HeLa cells indicate that menin, MLL, and RBBP5 co-localize with RNA polymerase II to transcriptional start sites, where histone H3 lysine 4 trimethylation occurs. Binding to the HOX clusters is strikingly different, with broad footprints that extend across inter- and intragenic regions of the HOX A and C clusters. To gain insights to the endocrine-specific tumor phenotype in MEN1, we compared these binding sites to those we mapped in primary human pancreatic islets, a common site of tumor formation in MEN1 patients. Menin was found to bind to a similar set of target genes in pancreatic islets, but with notable exceptions at the HOX clusters and elsewhere. Targets bound by menin exclusively in islet cells include key transcription factors involved in pancreatic endocrine development and insulin regulation. We suggest that absence of menin initiates tumorigenesis by mediating epigenetic changes in chromatin structure, potentially at the promoters of these key endocrine-specific genes.

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Expression profiling identifies similar expression pattern of uveal melanomas with chromosome 3 isodiosmy to those with monosomy 3. *M. Abdel-Rahman[1,2], Y. Yang[2], F. Davidorf[2], C. Eng[1].* 1) Clinical Cancer Genetics Prog, Ohio State Univ, Columbus, OH; 2) Department of Ophthalmology, Ohio Sate Univ, Columbus, OH.

Purpose: Monosomy of chromosome 3 (M3) is the most frequent somatic alteration in uveal melanomas (UMs). M3 has been suggested as a useful marker for detection of aggressive UMs. Contrary to earlier reports, recent studies indicate a high frequency of partial chromosome 3 deletion in UMs. Moreover, chromosome 3 isodisomy has been reported in up to 16% of UMs without M3. The aim of the study is to identify the expression pattern associated with chromosome 3 isodisomy and partial chromosome 3 alterations compared to M3 in UMs. Methods: We used a combination of comparative genomic hybridization (CGH), high resolution genotyping utilizing 38 microsatellite markers on chromosome 3 and gene expression profiling to study 13 primary UMs. The frequency of partial chromosome 3 alterations was studied in a total of 47 UMs. Results: 7/13 UMs with available CGH data and expression profiling showed M3. In addition 2/13 showed isodiomy of chromosome 3 indicated by minimal or no alteration of chromosome 3 by CGH and LOH of most of the markers on both chromosome 3 arms. The remaining 4/13 UMs showed partial gains and/or loss of chromosome 3. All samples with partial chromosome 3 alterations were confirmed by genotyping. Expression profiling identified similar expression patterns of samples with chromosome 3 isodisomy to those with M3. Expression profiling identified several regions of common decreased expression on both chromosome arms 3p and 3q. Finally we identified a high frequency (22/47) of UMs with partial chromosome 3 alterations. Conclusions: Our data have revealed similar expression patterns of UMs with chromosome 3 isodisomy to those with M3, suggesting that isodisomy 3 may portend similar poor prognosis as M3. These results also indicate that partial chromosome 3 alterations are under-diagnosed in UMs. The clinical significance of partial chromosome 3 alterations will need to be further explored. Taken together, our results are important in designing clinical diagnostic strategies for the detection of chromosome 3 alterations in UMs patients.

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Advanced genetic analyses on migraine: Trait component analysis and mouse model expression study. *M. Wessman[1,2], G. Oswell[3], M. Kallela[4], V. Anttila[1], M. Kaunisto[1], P. Tikka[1], E. Hämäläinen[1], D. Nyholt[5], J. Terwilliger[6], J. Kaprio[7], M. Färkkilä[4], L. Peltonen[8], A. Palotie[1].* 1) Finnish Genome Center and Dept of Clin Chem, Univ Helsinki, Finland; 2) Folkhälsan Research Center, Helsinki, Finland; 3) Dept of Pathology, UCLA, USA; 4) Dept of Neurology, Helsinki University Central Hospital, Finland; 5) Queensland Inst of Med Research, Brisbane, Australia; 6) Columbia Genome Center, Columbia University, NYC, USA; 7) Dept of Public Health, Univ Helsinki, Finland; 8) Dept of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland.

Migraine is a common chronic severe headache disorder with a strong genetic component and a prevalence of ~ 15% in the Caucasian population. We have collected nearly 700 multiple case families and based the diagnosis on the International Headache Society (IHS) criteria. This enables us to use medium-to-large size families for genome-wide linkage analysis and to perform case control association studies. Our previous genome-wide linkage study identified a locus on 4q24 linked to the endpoint diagnosis migraine with aura. We subsequently performed a trait component linkage analysis to test which IHS trait components provide best evidence of linkage to the 4q24 locus and whether additional susceptibility regions could be localized. Strongest evidence of linkage to 4q24 was achieved with unilaterality (parametric two-point LOD score 4.20), photophobia (3.73) and phonophobia (3.52). Pulsation was linked to 17p13 (4.65) and the IHS C-criteria to 18q12 (3.27). To identify positional candidate genes we performed whole transcriptome expression analysis with the Affymetrix 430 2.0 GeneChip on primary cultures of neurons and glial cells from the *tottering* mouse with a mutation in *Cacna1a*. Using a novel pathway identifying algorithm we identified 238 genes comprising 108 GeneOntology pathways as being differentially regulated including Ca^{2+} channel genes *Cacna1b* and *Cacng7*, Na^{+}/K^{+} pumps *Atp1a1* and *2*, *Atp1b1, 2* and *3*. We also identified several genes in our Chr 4 and Chr 17 restricted regions. Candidate genes identified in the mouse expression study are to be tested in our case control sample of 1800 subjects.

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A common non-synonymous SNP in the BRI3 (ITM2C) gene accounts for a significant portion of the linkage evidence for Parkinson disease on chromosome 2q. *N. Pankratz[1], L. Byder[2], C.A. Halter[1], A. Rudolph[3], C.W. Shults[4,5], P.M. Conneally[1], T. Foroud[1], W.C. Nichols[2], Parkinson Study Group-PROGENI Investigators.* 1) Medical & Molec Genetics, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Children's Hospital Research Foundation, Cincinnati, OH; 3) Univ Rochester, Rochester, NY; 4) Univ California, San Diego, CA; 5) VA San Diego Healthcare System, San Diego, CA.

Previously, we identified significant linkage to chromosome 2q35 using a sample of individuals with familial Parkinson disease (PD). The linkage was strongest among those families with verified PD and those with a stronger family history (four or more affected or an affected sibpair with an affected parent). The disease allele appeared to be inherited in an autosomal dominant fashion, and penetrance appeared to be high in those individuals with a strong family history. Subsequently, we have genotyped six additional markers in the linkage region. With these additional markers, the autosomal dominant LOD score for the subset remained significant, and the non-parametric linkage evidence of the verified PD sample increased from a LOD score of 2.5 to 4.0. One candidate gene in this region is BRI3 (ITM2C), which belongs to a family of integral membrane proteins. One of its homologs (BRI2) is associated with familial dementia. Little is known about BRI3, except that it is highly enriched in human brain and that it interacts with BACE1, an enzyme responsible for converting beta-amyloid, a protein involved in Alzheimer disease pathogenesis. When we screened our PD sample for mutations in the BRI3 gene, we identified a rare non-synonymous point mutation (Arg236Trp) in one of our patients, as well as a non-synonymous polymorphism (Gly53Ser) that appears to be common in the general population (allele frequency = 0.40). Using the Genotype-IBD Sharing Test (GIST) under the same hypothesis of autosomal dominant inheritance, we have shown that this common SNP accounts for a significant portion of the linkage to chromosome 2q (p=0.03). It is therefore possible that this polymorphism is a common PD susceptibility gene with low penetrance (similar to APOE for Alzheimer disease).

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High-density linkage screen in multiple sclerosis. *J. Haines, for the International Multiple Sclerosis Genetics Consortium.* Ctr Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN.

Ten centimorgan microsatellite map have been the standard tool used for whole genome linkage screening since the mid 1990s and to date 11 screens employing this methodology have been published in multiple sclerosis. Inspection of these data shows that average genotyping success and information extraction rates are disappointing (80% and 44% respectively). Moreover available evidence suggests that genotyping errors rates in these studies (particularly the older ones) are likely to be > 1% for many if not most of the markers typed. The advent of high throughput cost effective SNP genotyping has provided new whole genome linkage screening tools promising significantly improved genotyping success rates and accuracy, which at high density should substantially enhance information extraction. Prompted by the potential benefits of these new tools we have typed the Illumina BeadArray linkage mapping panel (version 4) in a set of 730 multiplex families from Australia (97), Scandinavia (165), the United Kingdom (298) and the United States (170), which together provide 945 affected relative pairs (830 sib pairs, 14 half sib pairs, 54 cousin pairs and 47 avunclular pairs). After excluding 474 markers with genotyping success rates of <98% and 776 markers found to be in linkage disequilibrium with flanking markers (with r2 > 0.16) data is available from 4506 markers spanning the genome. A total of 2709 samples have been typed. The mean information extraction is 79.3% (range 42.4 - 91.3%) and the observed Mendelian inconsistencies suggest that within this data the genotyping error rate is just 0.002%. Highly significant linkage is observed in the region of the MHC (MLS 11.7) while suggestive linkage is seen on chromosomes 17q23 and 5q33. This screen provides the most definitive linkage map for multiple sclerosis currently available and illustrates the substantial increase in power that can be achieved using high density SNP linkage mapping sets. It is clear that future attempts to identify non-MHC susceptibility genes in multiple sclerosis will have to involve large sample sizes and association based methodology.

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Homozygosity mapping with high-density SNP arrays identifies a novel gene for Bardet-Biedl Syndrome. *A.P. Chiang[1], J.S. Beck[1,2], A.L. Ferguson[1], K. Elbedour[3], R. Carmi[3], H.-J. Yen[1,2], M.K. Tayeh[1,2], J. Wei[1,2], D.Y. Nishimura[1], T.A. Braun[1], E.M. Stone[1,2], T.L. Casavant[1], V.C. Sheffield[1,2].* 1) University Iowa, Iowa City, IA, USA; 2) Howard Hughes Medical Institute, Iowa City, IA, USA; 3) Genetic Institute, Ben Gurion University of the Negev, Beer-Sheva, Israel.

The identification of mutations in genes that cause human diseases has largely been accomplished through the use of positional cloning, which relies on linkage mapping. In studies of rare diseases, the resolution of linkage mapping is limited by the number of meioses and marker density. One recent technological advance is the development of high density Single Nucleotide Polymorphism (SNP) microarrays for genotyping. The SNP arrays overcome low marker informativity by using a large number of markers to achieve greater coverage at finer resolution. To determine the utility of this technology for homozygosity mapping using small pedigrees, we genotyped a small consanguineous Israeli Bedouin (AT) family with autosomal recessive Bardet-Biedl Syndrome (BBS; obesity, pigmentary retinopathy, polydactyly, hypognoadism, renal and cardiac abnormalities, and cognitive impairment). While eight genes have been identified to cause BBS, over half of BBS patients have unknown genetic defects, leaving the possibility of additional genes, that when mutated, cause BBS. The AT family was a good candidate for homozygosity mapping with high density SNP arrays because it was not linked to any of the known BBS loci and a genome-wide scan with microsatellite markers at ~10 cM density did not reveal a linked locus. DNA from all four individuals affected with BBS from the family was hybridized to the HindIII chip of the Affymetrix 100K SNP chip set and the resulting data analyzed for regions of homozygosity. Regions of homozygosity were prioritized based on the physical distance, the number of consecutive homozygous SNPs, and marker density. Mutation analysis in the best candidate homozygosity region identified a gene with a conserved homozygous missense mutation. Functional analysis of this gene in a zebrafish system provides additional evidence that this is a BBS gene (BBS9).

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Genomeutwin-European twin cohorts: Combined analysis of genome scans guide to two QTL:s for body stature including one common Caucasoid X-chromosomal locus. *L. Peltonen[1], S. Sammalisto[1], T. Hiekkalinna[1], N. Martin[2], J. Harris[3], D. Boomsma[4], K. Christensen[5], K. Ohm Kyvik[5], N. Pedersen[6], T. Andrew[7], T. Spector[7], E. Widen[8], A. Palotie[8], M. Perola[1], GenomEUtwin.* 1) Dept Med Genetics & Molec Med, Univ Helsinki & Nat Pub Hth In, Helsinki, Finland; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Norwegian Institute of Public Health, Oslo; 4) Free University, Amsterdam, Netherlands; 5) University of Southern Denmark, Odense, Denmark; 6) Karolinska Institutet, Stockholm, Sweden; 7) St Thomas Hospital, London, United Kingdom; 8) University of Helsinki, Finnish Genome Center, Finland.

Twin cohorts provide a unique advantage for investigations of the role of genetics and environment in the etiology of common traits. Co-twins share environment throughout critical fetal period and early years of life and twin design harmonizes this component of complex traits in a unique manner. The EU-funded GenomEUtwin (www.genomeutwin.org) consortium consists of eight twin cohorts (Australian, Danish, Dutch, Finnish, Italian, Norwegian, UK and Swedish) with the total number of 600000 twin pairs. Federated database with open source code has been created to share the data across the cohorts. We performed QTL analysis of stature (body height) using genome-wide scans performed for 8775 individuals: Australia (n=3730), Denmark (628), Finland (772), Netherlands (1313), Sweden (102) and United Kingdom (2230). The marker maps were combined and related to the sequence positions using computer program developed by us which uses DeCode genetic map markers as an anchoring set (www.bioinfo.helsinki.fi/cartographer). We used the program Merlin for variance components analysis with age, sex and country-of-origin as covariates. The covariate adjusted heritability was 82% for stature in the pooled data set. We found evidence for two major QTLs for human stature on 15q24 (LOD=3.75, 1-lod drop 11cm) and Xq25 (LOD=2.73, 15cM). Especially the evidence for linkage for the X-chromosomal locus is contributed by most of the cohorts, thus suggesting an evolutionally old genetic variant having effect on the growth in European-based populations.

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Evidence for association between hepatic nuclear factor 4, alpha and triglycerides in Finnish familial combined hyperlipidemia families. *D. Weissglas[1], E. Suviolahti[1,2], J. Lee[1], A. Jokiaho[1], M-R. Taskinen[3], P. Pajukanta[1].* 1) Department of Human Genetics, UCLA, Los Angeles, CA; 2) Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland; 3) Department of Medicine, University of Helsinki, Finland.

Familial combined hyperlipidemia (FCHL), characterized by increased levels of serum total cholesterol, triglycerides (TGs) or both, is observed in up to 20% of patients with premature coronary heart disease. Previously we identified a locus linked to elevated TGs on chromosome 20q13.11 in Finnish FCHL families. Numerous linkage studies of type 2 diabetes mellitus (T2DM) have also found evidence of linkage to this region. Recently, several independent groups identified associations between single nucleotide polymorphisms (SNPs) in the hepatic nuclear factor 4, alpha (HNF4A) gene on 20q12-q13.1 and T2DM in Finnish and Ashkenazi Jews. Because there is a clear phenotypic overlap between FCHL and T2DM, we tested this gene region for association with FCHL in Finnish families. To date no association between plasma lipid levels and HNF4A has been identified in FCHL. We constructed the linkage disequilibrium (LD) structure of the HNF4A region by using both the HapMap data and recently published Finnish LD data. Based on that information, we genotyped the seven SNPs, previously associated with T2DM, and three additional tag-SNPs from distinct haplotype blocks to capture most of the genetic variation within the HNF4A gene, including promoters 1 and 2. A SNP in promoter 2 of the HNF4A gene was significantly associated with high TGs (P=0.007) in 720 family members of 60 FCHL families. Haplotype analyses also showed significant associations with TGs as well as with plasma apolipoprotein B and high-density lipoprotein levels for several HNF4A haplotypes. The most significant haplotype for high TGs (P=0.008) has a frequency of 0.289 in the Finnish FCHL families. In conclusion, this study is the first to provide significant evidence for association between the intragenic variants of the HNF4A gene and plasma lipid levels in FCHL.

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A new method to correct for population stratification in genetic case-control association studies: centralizing the non-central chi-square. *P. Gorroochurn[1], G.A. Heiman[2], S.E. Hodge[1,3], D.A. Greenberg[1,3].* 1) Division of Statistical Genetics,Dept Biostatistics, Columbia Univ, New York, NY; 2) Department of Epidemilogy, Columbia Univ, New York, NY; 3) Clinical-Genetic Epidemiology Unit, New York State Psychiatric Institute, New York, NY.

Recently, several authors have investigated the use of genomic information in an attempt to eliminate bias due to population stratification (PS) in case-control association studies. We here present a new method, the delta-centralization (DC) method, to correct for PS. DC works well even when there is a lot of bias due to PS. In the presence of PS, the usual chi-square statistics used to test for association have non-central chi-square distributions. Other methods approach the non-centrality indirectly, but we deal with it directly, by estimating the non-centrality parameter itself. Specifically: (1) We define a quantity delta, a function of the relevant subpopulation parameters, that exactly predicts (in relatively large samples) the elevation of the false positive rate due to PS, when there is no true association between marker genotype and disease. (Delta is quite different from F_ST and can be large even when F_ST is small.) (2) We show how to estimate delta, using a panel of unlinked neutral loci. (3) We show that the square of delta corresponds to the non-centrality parameter of the chi-square distribution. Thus, we can centralize the chi-square using our estimate of delta; this is the DC method. (4) We demonstrate, via computer simulations, that DC works well with as few as 25-30 unlinked markers, where the markers are chosen to have allele frequencies reasonably close (within 0.1) to those at the test locus. (5) We compare DC with genomic control and show that whereas the latter becomes over-conservative when there is considerable bias due to PS (i.e. when delta is large), DC performs well for all values of delta.

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A Spatial Clustering Approach to the Analysis of Genetic Association Studies. *E.R.B. Waldron[1], J.C. Whittaker[1,2], D.J. Balding[1].* 1) Epidemiology and Public Health, Imperial College London, London, United Kingdom; 2) Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London.

Haplotype-based approaches to the analysis of genetic association studies have important advantages over single-marker analyses, but they nevertheless suffer from limitations. For instance, rare haplotypes may not analysed effectively. Furthermore, the relationships between distinct but similar haplotypes that may share recent common ancestry are often not accounted for. We have developed an algorithm based on spatial statistics techniques, employing a simple distance in haplotype space that reflects evolutionary processes. The algorithm searches for case-rich clusters of similar haplotypes. Membership of this cluster corresponds to predicting the allele at an unobserved causal SNP. The algorithm can be applied to fine-mapping if the distance metric depends on the putative location of a causal allele. Using this algorithm to analyse data for the CYP2D6 gene, for which the true causal polymorphism is fully characterised, we correctly predicted nearly 98% of the genotypes at the major causal polymorphism and the functional mutation was mapped accurately. Simulation studies also revealed consistently better performance than alternative fine-mapping algorithms and allowed us to identify situations in which multi-point approaches offer a substantial improvement over single-point analyses.

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Admixture-Matched Case-Control Study: A Practical Approach for Genetic Association Studies in Admixed Populations. *J. Kho[1,2], H.J. Tsai[1,2], N. Shaikh[1,2], S. Choudry[1,2], M. Navqi[1,2], D. Navarro[1,2], H. Matallana[1,2], R. Castro[1,2], C. Lilly[3], H.G. Watson[4], K. Meade[5], M.L. Noir[6], S. Thyne[1], E. Ziv[1], E.G. Burchard[1,2].* 1) UCSF, San Francisco, CA; 2) Lung Biology Center, San Francisco General Hospital, SF, CA; 3) Brigham and Womens Hospital, Boston, MA; 4) The James A. Watson Wellness Center, Oakland, CA; 5) Children's Hospital and Research Institute, Oakland, CA; 6) Bay Area Pediatrics, Oakland, CA.

Case-control genetic association studies in admixed populations are known to be susceptible to genetic confounding due to population stratification. The transmission/disequilibrium test (TDT) approach can avoid this problem. But, the TDT is expensive and impractical for late-onset diseases. Case-control study designs, in which cases and controls are matched by admixture, can be an appealing alternative for genetic association studies in admixed populations. We applied this matching strategy when recruiting our African American participants in the Study of African American, Asthma, Genes and Environments (SAGE). Group admixture in this cohort consists of 83% African ancestry and 17% European ancestry. By carrying out several complementary analyses, our results show that there is substructure, but that the admixture distributions are almost identical in cases and controls, and also in cases only. We performed association tests for asthma-related traits with ancestry, and only found that FEV_1, a measure of asthma severity, was associated with ancestry after adjusting for socio-economic and environmental factors (p=0.01). We did not observe an excess of type I error rate in our association tests for ancestry informative markers and asthma-related traits when ancestry was not adjusted in the analyses. Furthermore, using the association tests between genetic variants in a known asthma candidate gene, $_2$ adrenergic receptor ($_2$AR) and FEF_{25-75}, a measure of bronchodilator drug responsiveness to the asthma medication, albuterol, as an example, we showed population stratification was not a confounder in our study. Our work demonstrates that admixture-matched case-control strategies can efficiently control for population stratification confounding in admixed populations.

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Analysis of fine structure recombination patterns in a human chromosomal region not previously known to harbor a recombination hot spot. *I. Tiemann-Boege[1], P. Calabrese[1], D.M. Cochran[1], R. Sokol[2], N. Arnheim[1].* 1) Molecular and Computational Biology Program, University of Southern California, Los Angeles, CA; 2) Health Science Center, University of Southern California, Los Angeles, CA.

For decades, evidence has suggested that crossing over is not homogenous across the human genome but can be highly localized in hot spots. Recent molecular studies of regions known to contain a hot spot based on classical methods have revealed that hot spots can be present where strong linkage disequilibrium (LD) is interrupted by patterns of very low LD. Whether recombination is generally restricted to hot-spots and whether hot spots can be found in larger chromosomal regions without exceptional frequencies of crossing over is yet to be addressed. Using sperm typing that selectively amplifies only recombinants in a pool of sperm genomes, we analyzed the recombination fractions in small contiguous intervals (~5 kb) of a 104 kb region of human chromosome 21 that showed no exceptional crossover activity. The observed recombination intensity ranges from 0.10 (95% confidence interval: 0.01, 0.29) to 12.21 (9.67, 15.05) times the human genome average rate. We identified two hot-spots with a recombination activity of 11.17 (9.98, 12.42) and 12.21 (9.67, 15.05) times the human average and four regions with a frequency ranging between 0.93 (0.59, 1.33) and 3.62 (3.09, 4.19) fold human average. The remaining regions all have an estimated frequency below 0.55 times the human average (all have a 95% upper bound below 0.84). The recombination patterns observed by sperm typing were compared with the patterns predicted by LDHat, Hotspotter and a third algorithm developed by one of us (P.C.). Patterns are roughly congruent with the recombination inferred from the population estimators with some exceptions. Our data suggests that it is likely that hotspots are usually found in larger chromosomal regions without exceptional frequencies of crossing over. Clearly, studies on other chromosomal intervals are needed to get a better genome-wide picture of the extent to which recombination is generally restricted to hot-spots and the degree to which LD analyses can predict recombination intensities.

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Improved Association Analyses of Disease Subtypes in Case-Parent Triads. *M.P. Epstein[1], I.D. Waldman[2], G.A. Satten[3].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept Psychology, Emory Univ, Atlanta, GA; 3) Centers for Disease Control and Prevention, Atlanta, GA.

The sampling of case-parent triads is an appealing strategy for conducting association analyses of complex diseases. In certain situations, one may have interest in using the triads to identify genetic variants that are associated with a specific subtype of disease, perhaps related to severity of symptoms or sensitivity to medication. A straightforward strategy for conducting such a subtype analysis would be to analyze only those triads with the subtype of interest. While such a strategy is valid, we show that triads without the subtype provide additional genetic information that increases power to detect association with the subtype. We incorporate this additional information using a likelihood-based framework that permits flexible modeling and estimation of allelic effects on disease subtypes and also allows for missing parental data. Using simulated data under a variety of genetic models, we show that our proposed association test consistently outperforms association tests that only analyze subtype triads. We also apply our method to a triad study of attention-deficit hyperactivity disorder and identify a genetic variant in the dopamine transporter gene that is associated with a hyperactive-impulsive subtype.

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Estimating genetic effects from genomewide association scan data. *S. Zollner, J. Pritchard.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Genomewide association studies are a powerful method to detect loci harboring variants that affect complex traits. After a successful scan for association it is of great interest to estimate the impact of a detected variant on the phenotype of a carrier (penetrance) and on the population as a whole (frequency). These estimates allow to assess the importance of a mutation, they may provide information about its biological effect and facilitate planning replication studies. As these estimations are usually performed based on the same data that were used to identify a variant, the results are affected by ascertainment bias, causing the genetic effects of a variant to be overestimated if not corrected for. This overestimation is considered the main reason that many replication studies fail as the sample size needed is underestimated. The magnitude of this bias depends on the power of the initial association study. Here, we present an approach that corrects for the ascertainment effect and generates an approximate maximum-likelihood estimate of the frequency of a variant and its penetrance parameters. The method produces both a point-estimate and a confidence region for all parameters. We apply this estimator to simulated datasets and study its performance and its ability to distinguish between different models of penetrance. We show that by taking epidemiological data into account, it is often possible to obtain fairly precise estimates of all parameters, even if the power of the genome scan is low. We also show that the error of the estimate decreases with sample size, independent of the power of the original test for association. Finally, we demonstrate that the same algorithm can be used to assess interactions between a genetic variant and environmental risk factors.

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Regression-Based QTL analysis method incorporating parent-of-origin effect. *O. Gorlova, L. Lei, D. Zhu, Y. Zhang, W. Li, S. Shete, R.A. Price, C. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

We present an extension of Sham et al.s (2002) regression-based quantitative-trait linkage analysis method to incorporate parent-of-origin effects. We separately regressed total, paternal, and maternal IBD sharing on traits squared sums and differences. We also developed a test for imprinting that indicates whether there is any difference between paternal and maternal regression. We use a panel of statistics to detect imprinting, which includes an overall T statistic (a test for total linkage), both parental T statistics (tests for parental-specific linkages), and the D index (a test for imprinting). We performed an extensive simulation to examine the performance of the panel. We found that when using empirical percentiles the method is very powerful in detecting parent-specific linkage with correct type I error rate for the non-linked parental component. Missing parental genotypes increase the type I error rate of both the linkage and imprinting tests and decreases the power of the imprinting test. When the major gene has low heritability, the power of the method decreases dramatically but the panel still performs well. We also used a permutation algorithm, which can ensure the appropriate type I error rate. We applied the method to a data from a study of 6 body size related measures and 23 loci on chromosome 7 for 255 nuclear families. Multivariate identities-by-descent were obtain using a modification of SIMWALK program. A parent-of-origin effect consistent with maternal imprinting was suggested at 99.67-111.26 Mb for body mass index, bioelectrical impedance analysis, waist circumference, and leptin concentration.

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Quantitative Trait Linkage Analysis using Gaussian Copulas. *M. Li[1], P. Song[2], G.R. Abecasis[1], M. Boehnke[1].* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Statistics and Actuarial Science, Univ Waterloo, Canada.

An important problem in gene mapping studies is to locate genetic variants that influence quantitative traits, such as cholesterol level, body mass index, and systolic/diastolic blood pressure. The quantitative traits can be interesting themselves or if they are closely related to diseases, because any quantitative trait locus (QTL) identified might also act as a disease susceptibility locus. Traditional QTL mapping uses the variance-components approach with the key assumption that the trait values in a family follow a multivariate normal distribution. Violation of this assumption often leads to an increase in false-positive linkage findings and biased parameter estimates. To accommodate non-normally distributed data, we describe a modified variance-components method that directly models the non-normal distribution, which we call the "Gaussian copula variance-components method". With Gaussian copulas, handling of a multivariate distribution can be separated into a marginal model and a model for the joint distribution of the transformed phenotype scores, where the dependence structure is characterized by genetic components. The Gaussian copula variance-components method allows the analysis of continuous, discrete, and censored data. In particular, the traditional variance-components method becomes a special case when the data are distributed as multivariate normal. Through the use of link functions, the Gaussian copula variance-components method can easily incorporate covariates. Our simulation results indicate that the proposed method yields unbiased parameter estimates and correct type I error rates for testing linkage with a variety of non-normal distributions. We also show that the proposed method provides a modest increase in linkage power as compared with the traditional variance-components method. The Gaussian copula variance-components method should provide a useful arsenal of tools for the mapping of genes that influence non-normally distributed quantitative traits.

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Extension of Variance Components Approach to Incorporate Repeated Measurements. *L. Liang, W. Chen, G. Abecasis.* Biostatistics, University of Michigan, Ann Arbor, MI.

When subjects are measured multiple times, it is important for a linkage analysis to appropriately take into account these repeated measures. In this study, we extend the variance components approach to model repeated measures in a quantitative trait linkage study. We show for the case of a balanced design where the same number of measurements is taken for each subject, a standard linkage test that takes the average of measures as the trait of interest is identical to the linkage test based on our extension of the variance components model. We give the general formulas of optimal sample size and number of repeated measures for a given power or cost. We carry out analytical calculations and perform simulations to compare power for different sample sizes and number of repeated measures across several scenarios (varying distance between markers from ~0 to ~10 cM, considering SNP and microsatellite markers, and varying major gene heritability, total heritability and measurement error from 2% to 20%, 8% to 80% and 0% to 60% of trait variability, respectively). We find that repeated measures provide substantial power improvements across genetic models. The proportional increase in expected LOD score depends mostly on measurement error and total heritability but not much on marker map or number of alleles per marker. For example, when measurement error takes up 20% of the trait variability and 4 measures per subject are taken, the proportional increase in expected LOD score ranges from 38% for trait with low heritability to 63% for trait with high heritability. When 2 measures per subject are taken, the increase ranges from 23% to 36%. For unbalanced designs, we use simulations to compare power for different sampling schemes. Finally, we give recommendations on whether to take repeated measures or to recruit additional subjects for different levels of measurement errors and ratios of genotyping, subject recruitment and phenotyping costs.

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Methods for detecting gene-gene interaction in extended pedigrees. *B.S. Maher, T. Goldstein McHenry, R.J. Weyant, M.L. Marazita.* Univ Pittsburgh, Pittsburgh, PA.

Several approaches to detecting statistical non-independence between unlinked loci, with respect to a phenotype (gene-gene interaction), in a linkage framework have been proposed. Few of those approaches are applicable to multiplex extended pedigrees. We examined the power of four approaches: Nonparametric linkage score (NPL) correlation (NPLC; Cox et al 1999), NPL regression (NPLR; Langefeld & Boehnke 1999), Ordered Subset Analysis (OSA; Hauser et al 2004) and the extended conditional logistic model (CLA; Olson 1999). NPLC, NPLR and OSA are especially useful when a locus of interest due to a linkage signal has been identified but a candidate gene (marker) has not yet been characterized. NPLC examines the correlation between per family nonparametric linkage statistics at unlinked regions of the genome. NPLR is an extension of the NPL statistic that allows for the simultaneous testing of multiple loci and the interactions between them in a matched case-control conditional logistic framework. OSA is an approach to linkage analysis where evidence for linkage at one locus is maximized over subsets of families rank-ordered by evidence for linkage at another. CLA can be used to test the interaction between a candidate marker, included as a covariate, and unlinked regions of the genome. Importantly, each of these approaches tests for interaction beyond the marginal effects at each locus by examining either the LOD difference between models with and without interactions (OSA and CLA) or by examining the relationship between the linkage statistics at unlinked regions (NPLR and NPLC). To test the power of the methods and their ability to distinguish epistasis from heterogeneity, we simulated datasets consisting of 500 multiplex pedigrees under four different disease models with varying degrees of interaction. To address concerns regarding the behavior of the CLA test statistic, genomewide simulations were performed to determine the null distribution. Each approach achieved its best power under the Dominant-Dominant model. CLA had substantially more power than the other approaches, but is limited in that it relies on prior identification of a candidate marker.

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A Bayesian model averaging approach to the analysis of large-scale genetic association studies allowing for two-locus epistasis. *A. Morris, L.R. Cardon.* Wellcome Trust Ctr Human Gen, Oxford, United Kingdom.

It is widely accepted that population-based association studies have the potential to locate genes contributing effects to complex traits. Large-scale studies of thousands of single nucleotide polymorphisms (SNPs) are becoming increasingly feasible as a result of improvements in the efficiency of genotyping technology and a better understanding of the extent and distribution of linkage disequilibrium (LD) throughout the genome. However, despite these advances, the issue of how best to analyse the data from large-scale association studies remains unresolved.

There is increasing evidence from model organisims to suggest an important role for epistasis in the aetiology of complex traits. The obvious approach is thus to test all possible pairs of SNPs for association. Despite the substantial penalty incurred for multiple testing, this strategy has been shown to be more powerful than a single-locus search, unless there are strong marginal effects. Alternatively, standard statistical variable selection techniques can be employed to identify the most important marginal effects and pair-wise interactions. However, this approach does not take account of uncertainty in the underlying genetic model of association, leading to inflation of false positive error rates.

Here, a novel application of Bayesian model averaging (BMA) techniques to the analysis of large-scale genetic association studies is presented. The underlying genetic model is treated as unknown, but could include marginal effects at each SNP, and interactions between each pair of loci to allow for epistasis. Markov chain Monte Carlo methods are used to sample from the posterior distribution of genetic models, and can be used to approximate the posterior probability of association of each SNP with the trait under investigation. The results of a detailed simulation study suggest BMA outperforms testing all possible pairs of SNPs except for models of "pure" epistasis with no marginal effects.

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Detecting identical-by-descent DNA intervals between affected distant relatives using high-density SNP genotyping. *S. Nelson[1], B. Merriman[1], Z. Chen[1], J. Jen[2].* 1) Dept. of Human Genetics, University of California, Los Angeles, CA; 2) Dept. of Neurology, University of California, Los Angeles, CA.

Recent advances in the parallel typing of SNPs using high density oligonucleotide arrays provides a powerful tool for determining association with common alleles and linkage within pedigrees. Here we introduce a general approach to mapping a risk locus inherited from a founder, using high density SNP typing of only the affected pedigree members most distantly related through the founder. Using data from genome-wide 10K, 120K, and 500K SNP arrays, we have developed a statistical model for assessing the likelihood that genomic DNA segments are identical-by-descent (IBD) in a pair of distant relatives. We show that ~150 consecutive, independent SNP genotypes are sufficient to clearly identify intervals as IBD on one chromosome between two related individuals. The method is especially well suited to detecting IBD fragments that would exist in affected pairs in the present population that trace back to a risk allele founder 3100 generations removed. To demonstrate the approach, a previously mapped autosomal dominant mendelian disorder was mapped to the appropriate single location in the genome by typing distant relative pairs on 50K SNP arrays. Family relationships were not used in the analysis, and just three affected individuals were sufficient to map to a 14 Mb locus. This method provides a robust and powerful means to detect linkage using dense SNP maps and greatly reduces the number of typed individuals needed to determine linkage. Thus, we show that the detection of specific segments of IBD sharing between distant relatives is an effective approach for the rapid mapping of rare disease loci. By extension, we also expect it to be useful for mapping of complex traits in which risk alleles originate from ancient founders, even if the specific distant relationships are unkown in the affected population being studied.

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Strategies for Detecting Epistasis via Genome-Wide Association. *D. Evans, M.S. Taylor, J. Marchini, A.P. Morris, L.R. Cardon.* Dept Statistical Genetics, Wellcome Trust Ctr, Oxford, United Kingdom.

Studies in model organisms suggest that epistasis may play an important and ubiquitous role in the etiology of complex diseases and traits in humans. With the era of large scale genome-wide association studies approaching, it is important to quantify whether it will be possible to detect statistical epistasis using realistic sample sizes in humans, and to what extent undetected epistasis will adversely affect power to detect association when single locus approaches are employed. We investigated the power to detect association for a range of two-locus quantitative trait models which incorporated varying degrees of epistasis. We evaluated the power to detect association using a single locus model which ignored interaction effects, a full two-locus model which allowed for interactions and a two-stage strategy whereby a subset of loci initially identified using a single locus test were analyzed using the full two-locus model. Despite the penalty introduced by multiple testing, fitting the two-locus model performed better than single locus tests for many of the situations considered, particularly when compared with attempts to detect both individual loci. The power of the two stage strategy depended on the first stage cut-off, the combination of allele frequencies at the loci and on the underlying model responsible for the interaction. An appropriate cut-off level increased power to detect association, whereas too strict a cut-off risked discarding target loci and too liberal increased the penalty due to multiple testing. Optimal conditions for this trade-off were model dependent and related to allele frequencies. We supplement the theoretical work with actual data from a genome-wide scan of gene expression levels for which we have found evidence of epistasis. Expression levels of IRF5, a gene implicated in Systemic Lupus Erythematosus, were associated with a SNP located in the IRF5 gene, and another SNP located close by RANKL, a gene involved in the same signaling pathways. Our results suggest that it is feasible to detect interacting loci which contribute to moderate proportions of the phenotypic variance using realistic sample sizes with appreciable power.

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A Coalescent-based Marker Selection Tool for Association Studies. *E.F. Tsung, G. Marth.* Department of Biology, Boston College, Chestnut Hill, MA.

The International HapMap project is genotyping millions of SNPs in hundreds of individual reference samples from different world populations. This data set will enable the annotation of allelic association patterns within the reference samples in local genome regions or along entire chromosomes. The genotype data and the annotations will collectively form a large resource to aid marker selection for clinical case-control association studies. However, the utility of the markers selected based on the HapMap reference samples depends on the degree to which these patterns remain constant across other sets of samples such as those from clinical populations. This question may be studied experimentally, by genotyping additional individuals, and comparing the strength of association in these samples to the HapMap reference samples.

Here we describe a computational alternative to costly genotyping of such additional samples. First, using a Coalescent technique, we generate computational haplotypes where a subset of these recapitulates the allele structure observed in the HapMap reference samples, and another subset is used as a proxy for additional samples such as those that will be used in a future case-control association study. We use these computational samples to optimize SNP marker selection. The advantage of this approach is that marker selection is based not only on the HapMap reference samples, but also on a computational representation of the larger target population in the study.

The performance of this approach will depend on how well our Coalescent computational samples approximate the haplotype structure of actual samples. To assess this, we will test our method under various marker distances, recombination rates, and perturbations in demographic model parameters. Furthermore, we will compare at available regions of the genome and at the same marker locations present in HapMap, our computational samples to genotype data collected from non-HapMap samples. Our method, combined with current tag SNP selection techniques, will form the basis of an interactive software tool to aid marker prioritization for disease association studies.

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Fukutin interacts with and modulates POMGnT1. *H. Xiong[1], K. Kobayashi[1], M. Tachikawa[1], H. Manya[2], T. Chiyonobu[1], Y. Nagai[1], T. Endo[2], T. Toda[1].* 1) Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, Osaka, Japan; 2) Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

Recent identification of mutations in certain genes encoding proteins with demonstrated or putative glycosyltransferase has revealed a novel mechanism for congenital muscular dystrophy. Fukuyama congenital muscular dystrophy (FCMD), muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS) and Large^{myd} mice all are characterized by hypoglycosylated -dystroglycan (-DG), as evidenced by a ~60kDa reduction in the relative molecular weight of -DG. However, only POMGnT1 (MEB) and POMT1 (WWS) are known to possess intrinsic glycosyltransferase activity. Here we show that fukutin lacks the enzymatic activity needed for *O*-mannosyl glycosylation of -DG but co-localizes with POMGnT1 in the Golgi apparatus. Fukutin and POMGnT1 (but not LARGE) co-immunoprecipitated from transfected cell lines and an *in vitro* binding assay. Two-hybrid analysis confirmed the fukutin-POMGnT1 interaction. When co-expressed with the ER-localized Y371C mutant fukutin, POMGnT1 relocated from the Golgi to the ER. The transmembrane domain of fukutin mediates its localization to the Golgi apparatus and participates in the interaction with POMGnT1. Finally, we demonstrate that POMGnT1 enzymatic activity in knock-in mice carrying the human retrotransposal insertion in the 3 noncoding region of *fukutin* decreased approximately 30% relative to wild type controls. From these findings, we suggest that fukutin forms a complex with POMGnT1 and modulates its enzymatic activity.

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Deficiency of a Neuron-specific Isoform of the TAF1 Gene Is Associated with X-linked Dystonia-Parkinsonism. *S. Makino[1], S. Ando[1], M. Tomizawa[2], H. Ando[2], S. Goto[3], S. Matsumoto[1], D. Tabuena[4], E. Maranon[4], M. Dantes[5], L.V. Lee[5], K. Ogasawara[6], I. Tooyama[7], H. Akatsu[8], M. Nishimura[1], R. Kaji[1], G. Tamiya[1].* 1) Department of Neurology, Tokushima Univ Sch Medicine, Tokushima, Japan; 2) Department of Molecular Life Science, Tokai Univ Sch Medicine, Kanagawa, Japan; 3) Department of Neurosurgery, Sch Medical Sciences, Kumamoto University, Kumamoto, Japan; 4) Department of Neurology, West Visayas State University Hospital, Iloilo, Panay, Philippines; 5) Department of Health, Philippine Children's Medical Center, Quezon Avenue, Quezon City, Philippines; 6) Department of Pathology, Shiga Univ Medical Science, Otsu, Japan; 7) Molecular Neuroscience Research Center, Shiga Univ Medical Science, Otsu, Japan; 8) Choju Medical Institute, Fukushimura Hospital Toyohashi, Aichi, Japan.

X-linked recessive dystonia-parkinsonism (XDP; OMIM#314250) is a sex-linked recessive movement disorder endemic to the Philippines, with pathological similarities to Huntington disease (HD; OMIM+143100). The disease gene, *DYT3*, has been mapped to Xq13.1. In a search for the causative gene, we performed genomic sequencing analysis based on the shotgun strategy using a BAC contig constructed from genomic DNA of an XDP patient, followed by expression analysis using the patient'fs brain tissue. We found a disease-specific SVA retrotransposon insertion in an intron of the *TAF1* (TATA-binding protein-associated factor 1) gene, which encodes the largest component of the TFIID complex, and significantly decreased expression levels of a neuron-specific isoform of *TAF1* in the caudate nucleus. We also identified an abnormal pattern of DNA methylation in the retrotransposon and its surrounding region in the genome, which could account for decreased expression of the isoform. The *TAF1* gene may have a tissue-specific role in RNA polymerase II-mediated expression of genes that are essential for caudate neurons, such as the dopamine receptor D2 (*DRD2*) gene, whose expression is also decreased in HD. XDP and HD, together with other neurological diseases associated with transcriptional dysregulation, may comprise an expanded spectrum of transcription syndromes.

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Generation and characterization of a mouse model of paroxysmal non-kinesigenic dyskinesia. *J. Nakayama, Y. Xu, H.Y. Lee, R. Tu, M. Vo, P.D. Ptacek, J. Cheung, Y.H. Fu, L.J. Ptacek.* Department of Neurology, University of California San Francisco, San Francisco, CA.

Paroxysmal non-kinesigenic dyskinesia (PNKD) is an autosomal dominant episodic movement disorder characterized by spontaneous attacks of dystonia, chorea, and athetosis begin in childhood through early adulthood. The attacks are precipitated by alcohol, coffee, stress and fatigue. PNKD has been mapped on chromosome 2q33-q35, and two mutations in the myofibrillogenesis regulator 1 gene (MR-1) were recently identified in PNKD families. The mutations cause changes (Ala to Val) in the N-terminal region of two MR-1 isoforms. The MR-1 gene function is unknown, and it is still not understand how mutations in this gene lead to manifestation of the PNKD phenotype. In order to elucidate the function of the MR-1 protein, we have begun developing animal models. We created transgenic mice with a mouse BAC clone (RP24-112K19) containing the wild-type murine ortholog (mMR-1). This BAC clone was modified to introduce the Ala7Val and Ala9Val mutations. Analysis of transgene copy number showed that two lines contained 2-3 copies and one line had one copy. Transgene expression in these mice were confirmed by RT-PCR. These mice were subsequently bred with C57/Bl6 mice. Transgenic mice showed normal development and their neurological reflexes, as assessed thus far, are normal. The body weight in transgenic mice was significantly lower (10-15%) than their wild type littermates. Transgenic mice appear to recapitulate the human phenotype as they have dystonic attacks precipitated by caffeine and ethanol. Transgenic mice from all three lines showed these attacks. We also generated mMR-1 knockout mice. Homozygous knockout mice were viable and reproductive, and had no gross phenotypic abnormalities. They did not show attacks by either caffeine or ethanol. Our results suggest that the gain-of-function mutations in MR-1 might be responsible for the PNKD phenotypes. Using the PNKD mouse model, we hope to elucidate the function of the MR-1 protein and understand the pathophysiology of this disorder.

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Neuropathy target esterase gene mutations cause motor neuron disease. *S. Rainier[1], M. Bui[1], L. Ming[1], E. Plein[1], D. Thomas[1], D. Tokarz[1], C. Delaney[1], J.W. Albers[1], R.J. Richardson[1,2], J.K. Fink[1,3].* 1) Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109; 2) Toxicology Program, University of Michigan, Ann Arbor, MI 48109; 3) Geriatric Research, Education and Clinical Center, Ann Arbor Veteran's Affairs Medical Center,Ann Arbor, MI 48109.

We evaluated two unrelated kindreds in which affected subjects exhibited autosomal recessive, slowly progressive lower extremity spastic weakness associated with wasting of distal upper and lower extremity muscles. Subjects met clinical criteria for amyotrophic lateral sclerosis (ALS) and conformed to Troyer syndrome. Magnetic resonance imaging demonstrated spinal cord atrophy and electromyography identified motor axonopathy. Genetic analysis of one consanguineous kindred suggested linkage between the disorder and a 22 cM locus on chromosome 19p13, a region containing neuropathy target esterase (NTE). Analysis of NTEs coding sequence in this family showed an M1012V mutation disrupting an inter-species conserved residue in NTEs catalytic domain in both affected individuals but not unaffected individuals or 98 control subjects. Analysis of NTEs coding sequence in the second non-consanguinous kindred showed that affected subjects were compound heterozygotes for two NTE mutations: one allele had 2826A>G mutation which causes substitution of an inter-species conserved residue R890H in NTEs catalytic domain; the other allele had a 4 bp insertion (NTE mRNA position 3104) which causes protein truncation after residue 1020. These mutations were present separately in each carrier parent and absent in 98 control subjects.

Discovering that NTE mutations cause corticospinal and peripheral motor axon degeneration in these families indicates the importance of the NTE pathway in maintaining axonal integrity; raises the possibility that NTE pathway disturbance contributes to other motor neuron disorders including ALS and primary lateral sclerosis; and supports the role of NTE in the pathogenesis of organophosphorous compound-induced delayed neurotoxicity. Understanding the pathogenesis of NTE-mutation motor neuron disease will provide insight into the treatment of this and other motor neuron diseases.

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A high-throughput metabolomics-guided screening of ENU mice for mouse models of human metabolic diseases: identification of mouse model of LCHAD deficiency. *J.Y. Wu[1], H.J Kao[1], C.C. Huang[1], R. Stevens[2], D. Millingon[2], Y.T. Chen[1, 2].* 1) Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan, Taiwan; 2) Dept of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA.

Our laboratory has long interests in rapid analysis of metabolites at the whole-organism level (a metabolomics approach). We previously reported the use of tandem mass spectrometry screening for amino acid and acyl carnitine profiles to detect the derangement in the pathways of amino acid and fatty acid metabolism in mice treated with the N-ethyl-N-nitrosourea (ENU) and identified a mouse model resembling human maple syrup urine disease (J Clin Invest 113: 434, 2004). With only 25 l of whole blood spotted on the Guthrie card, this method could detect more than 20 metabolic diseases with defect in amino acid and fatty acid metabolism. Here we report, in a recessive screening of 2,300 G3 mice from 61 families, we identified mice with elevated blood long chain acyl carnitines (C16-OH, C18-OH, and C18:1-OH carnitines). Those mice had poor weight gain but survived to adulthood despite a shorter life span (affected died at age 12 to 18 months as compared to > 24 months for non-affected sibs). The elevation of long chain acyl carnitines reappeared in the F2 generation confirms its heritability. Affected mice were outbred to C3H for disease gene mapping. A panel of 299 SNPs was used for whole genome SNP homozygosity mapping. Genotyping results (performed on the MassARRAY SNP genotyping platform) showed homozygosity in 5 consecutive SNPs on chromosome 5 spanning from 34 Mb to 63 Mb where candidate genes, Hadha and Hadhb, coding for mitochondria trifunctional proteins and subunit, locate nearby. Direct sequencing identified a nucleotide T to A transversion in exon 14 (c.1210T>A) of Hadhb which resulted in a missense mutation of methionine to lysine (M404K). This is the first viable late-onset animal model of LCHAD (Long-Chain 3-HydroxyAcyl-CoA Dehydrogenase) deficiency due to Hadhb mutation. These mice could provide an important animal model for study of long chain fatty acid metabolism, investigating the pathogenesis of LCHAD deficiency and development of more effective therapies for this disease.

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PGC-1-alpha transcription interference produces deranged thermoregulation in Huntingtons disease transgenic mice. *P. Weydt, V.V. Pineda, S. Luquet, R.T. Libby, A.R. La Spada.* Lab Medicine, Univ Washington, Seattle, WA.

Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by motor and cognitive impairment. The motor abnormality stems from loss of medium spiny neurons in the striatum. HD is caused by a CAG repeat expansion in the huntingtin gene, and is thus one of 9 neurodegenerative disorders due to polyglutamine (polyQ) tract expansions. An intriguing feature of HD is enhanced vulnerability of the striatum to neurodegeneration. As part of a baseline evaluation of the HD N171-82Q mice, we measured core body temperature in HD mice. We found that at ~135-140 days of age, all HD transgenic mice (but no controls) developed progressive hypothermia with core body temperatures of < 35C. With progression of motor symptoms and weight loss, HD N171-82Q mice displayed profoundly deranged thermoregulation, with core body temperatures dropping to < 30C. We then subjected HD mice and controls to a 4C cold challenge, and documented a profound adaptive thermogenesis defect, as HD mice are unable to maintain core body temperature from 60 min into the cold challenge ($p < .05$). As body temperature regulation in rodents involves PPAR co-activator 1 (PGC-1) up-regulation of UCP-1 in brown fat, we dissected the brown fat of HD mice and found it to be abnormal. We then measured PGC-1 levels in brown fat from HD mice subjected to cold challenge, and found it to be appropriately up-regulated. UCP-1 mRNA and protein levels, however, were not increased in the brown fat of HD mice. We co-transfected 3T3-L1 cells with PPAR, RXR, the UCP-1 promoter reporter construct, and exon1/2 htt 24Q or 104Q expression constructs. We found that htt 104Q markedly suppressed UCP-1 promoter reporter expression in cells treated with ligand, but that co-transfection of the PGC-1 expression vector rescued htt 104Q repression ($p < .001$). Our results indicate that polyQ-expanded htt is interfering with PGC-1-mediated up-regulation of UCP-1 expression, and suggest a role for transcription interference with PGC-1-regulated mitochondrial biogenesis and metabolic pathways in HD. Impairment of PGC-1 function may thus account for the cell-type specificity in HD.

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Spectrin mutations cause spinocerebellar ataxia type 5. *L.P.W. Ranum[1,2], K.A. Dick[1,2], M.R. Weatherspoon[1,2], J.C. Dalton[1,2], G. Stevanin[5], A. Dürr[5], C. Zuehlke[6], K. Buerk[7], A. Brice[5], H.B. Clark[3,4], L.J. Schut[8], J.W. Day[2,3], Y. Ikeda[1,2].*
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Spinocerebellar ataxia type 5 (SCA5) is an autosomal dominant disorder characterized by incoordination, eye movement abnormalities and slurred speech. MRI findings show dramatic cerebellar cortical atrophy, with the brainstem and other regions of the brain appearing normal. We previously mapped SCA5 to chromosome 11q13 in an 11-generation American kindred. Subsequently, two additional SCA5 families from France and Germany were reported.

We have now discovered that beta-III spectrin (*SPTBN2*) mutations cause SCA5. The American and French families have separate in-frame deletions in the third of 17 spectrin repeats. Consistent with Purkinje cell degeneration in SCA5, beta-III spectrin transcripts are abundantly expressed in brain, with relatively high expression in cerebellar Purkinje cells. RT-PCR analysis of cerebellar autopsy tissue from an affected member of the American family indicates that both the normal and deleted forms of beta-III spectrin transcripts are expressed in affected tissue. Western analysis of cerebellar tissue shows dramatic solubility differences of EAAT4, a glutamate transporter and Purkinje cell specific protein normally stabilized by beta-III spectrin. A third mutation in the German family, located in the dynactin-binding site, and distribution differences in Purkinje cell proteins (EAAT4, GluR2, calbindin, parvalbumin) from SCA5 autopsy tissue, suggest disruptions in vesicle transport. Spectrin mutations are a novel cause of neurodegenerative disease that possibly affect glutamate signaling and vesicle trafficking, pathways previously implicated in Alzheimer and Huntington diseases, SCA1 and ALS.

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Targeted deletion of the Sca8 ataxia locus in mice causes abnormal gait, progressive loss of motor coordination and Purkinje cell dendritic deficits. *Y. He, K.A. Benzow, H.B. Clark, M.D. Koob.* IHG and LMP, U of MN, Minneapolis, MN.

Spinocerebellar ataxia type 8 (SCA8) patients typically have a slowly progressive, adult-onset ataxia that is accompanied by significant cerebellar atrophy. SCA8 is caused by large CTG repeat expansions in the untranslated antisense RNA of the Kelch-like 1 gene (KLHL1), but the precise relationship between the SCA8 CTG expansion and the disease status of SCA8 patients is complex and is still poorly understood. In order to more fully characterize the underlying molecular mechanisms involved in SCA8, we have developed a mouse model in which Klhl1 is deleted in either all tissues or is deleted specifically in Purkinje cells only. We found that mice that were homozygous for this deletion had a significant loss of motor coordination as measured by performance on an accelerating Rotarod by 24 weeks of age, and that the performance of mice assessed at 12 weeks or younger did not significantly differ from controls. Mice with KLHL1 specifically deleted in only Purkinje cells had a loss of motor coordination that was almost identical to mice with complete KLHL1 deletions. We also analyzed the gait of mice with homozygous KLHL1 deletions by detailed computer analysis of treadmill gait (Treadscan apparatus) and found that the mice develop significant gait abnormalities by 10 weeks of age. Interestingly, we found that mice that were heterozygous for the KLHL1 deletion had gait abnormalities that also significantly differed from controls. Finally, we found significant Purkinje cell dendritic deficits, as measured by the thickness of the molecular layer, in all mice in which KLHL1 was deleted (both total and Purkinje cell specific) and an intermediate reduction in molecular layer thickness in mice with reduced levels of KLHL1 expression (heterozygous deletions). The results from this mouse model are consistent with a role for KLHL1 as an actin-organizing protein that is essential for establishing or maintaining the dendritic structure and function in Purkinje cells and indicates that loss of KLHL1 activity is likely to play a significant part in the underlying pathophysiology of SCA8.

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Molecular and clinical aspects of myotonic dystrophy: seven yearsexperience of direct molecular testing on 2650 Italian DM cases, risk prediction for clinical phenotype on the basis of molecular data. *L. Baghernajad Salehi[1], E. Bonifazi[1], M. Gennarelli[2], A. Botta[1], L. Vallo[1], R. Iraci[1], A. Nardone[1], A. Pietropolli[1], E. Di Stasio[3], G. Novelli[1].* 1) Biopathology, Tor Vergata University, Rome, Italy; 2) IRCCS, Brescia, Italy; 3) UCSC, Rome,Italy.

Myotonic dystrophy type 1(DM1;OMIM #160900)is an autosomal-dominant genetic disorder with multisystemic clinical features associated with a CTG expansion in 3'UTR of the myotonic dystrophy protein kinase gene (DMPK) on chromosome 19q13.3. Subject showing less than 35 CTG can be consider normal and subjects showing from 35 to 50 CTG are defined premutated.DM1 genotypes can be divided on the basis of repetition number into E1(from 50 to 200 CTG),E2(from 200 to 1000 CTG) and E3(1000 CTG)classes.We report on a 7 years experience in providing symptomatic and prenatal molecular diagnostic services for MD over the Italian territory on 2650 symptomatic patients and 185 prenatal diagnoses.Molecular analyses were informative in all examined cases for the detection and characterization of the CTG expansion.The most frequent expanded alleles in Italian patients were in the range of 200-1000 CTG repeat (E2 class).In order to study the intergenerational CTG transmission, we subdivided the data into 3 groups: an increase of the CTG expansion from E1 to E2 class, an increase of the CTG expansion from E2 to E3 class and finally an higher increase from E1 to E3 class.In group E1-E2 anticipation is more frequent in father-child pairs whereas in the E2-E3 group anticipation is more frequent in mother-child pairs (because of DM1 male infertility or sperm selection).There is no significant difference between the distribution of contraction events within the different classes and among parental transmission.A genotype-phenotype correlation was performed in order to assess the genomic risk to manifest more than one clinical feature.We were able to demonstrate that E2 class shows an increased risk to develop more clinical complication with an OR of 8.8. Our experience has confirmed that direct molecular analysis for the myotonic dystrophy mutation has proved to be of considerable value in diagnosis and phenotype prediction.

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Error-prone repair of slipped CAG/CTG repeats by human neuron proteins leads to trinucleotide expansions.

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Instability of trinucleotide repeats, the causative mutation of ~30 neurodegenerative/neuromuscular diseases, is thought to involve escaped repair of slipped-strand DNA intermediates. We report the first characterization of the fidelity and efficiency of slipped CAG/CTG processing by human proteins, a process that is pivotal to understanding the mechanism of trinucleotide repeat instability. Using human cell extracts and DNAs with slip-outs of (CAG)20 or (CTG)20 we observed an hierarchy of repair outcomes. A new form of error-prone repair was detected, whereby excess repeats were incompletely excised, presenting a novel path to generate expansions, but not deletions. Both the improved correction of deletion intermediates over expansion intermediates, as well as the retention of excess repeats in error-prone repair products may be sources for the expansion bias in humans. Neuron-like cell extracts yielded each repair outcome, supporting a role for these processes in CAG/CTG instability in patient post-mitotic brain cells. Curiously, expansion mutations of CAG/CTG transgenes in four transgenic mice required functional mismatch repair proteins MSH2 and MSH3; a mutagenic role which contrasts with the corrective repair role played by these proteins against other mutations. Mismatch and nucleotide excision repair proteins hMSH2, hMSH3, hMLH1, XPF, XPG or polymerase beta were not required - indicating that their role in instability may precede that of slip-out processing. We propose that through DNA-binding MMR proteins may drive expansion mutations by forming slipped DNA mutagenic intermediates. Differential processing of slipped-repeats may explain the expansion bias and different mutation patterns occurring between various disease loci or tissues including brains.

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Deficiencies in different subunits of the Conserved Oligomeric Golgi (COG) complex define a novel group of Congenital Disorder of Glycosylation. *G. Matthijs[1], F. Foulquier[1], E. Vasile[2], D. Ungar[3], M. Krieger[2], W. Annaert[4].*
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Congenital Disorders of Glycosylation (CDG) are a group of rare, genetic disorders characterized by defects in one of the many enzymes, transporters or other functional proteins, required in the glycosylation pathway. Up to now, more than 15 defects have been described, mainly in the endoplasmic reticulum (Type I) and in the Golgi compartment (Type II). Whereas these CDG cases have defects in genes encoding proteins directly involved in the glycosylation process, recent work has shown that the abnormalities may also be due to abnormal intracellular trafficking of resident Golgi enzymes or transporters involved in glycosylation. Indeed, the function of the Golgi being dependent of the correct localization of all glycosylation enzymes and other resident proteins. This process is achieved by a careful balance between of forward and retrograde traffic of cargo and resident proteins. The Conserved Oligomeric Golgi (COG) complex which is a heterooligomeric tethering complex plays a key role in the retrograde vesicular transport. Two CDG-II patients with two different COG deficiencies (COG1 and COG8) were found. They have a multi-system disease with severe neurological involvement. Both of them have a combined N- and O-glycosylation defect characterized mainly by hyposialylation. The mutations destabilize other COG subunits, and affect the subcellular localization and hence the overall integrity of the COG complex. It further suggest that the interaction between COG1 and COG8 play a central role in the formation and the functionality of the whole COG complex. Deficiencies in this complex thus define a new group of glycosylation disorder.

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Identification of the first human mutations in a component of the selenocysteine incorporation machinery. *A.M. Dumitrescu[1], X.H. Liao[2], M.S.Y. Abdullah[4], J. Lado-Abeal[2], F. Abdul Majed[5], L.C. Moeller[2], G. Boran[6], R.E. Weiss[2], S. Refetoff[2,3].* 1) Human Genetics, Univ. of Chicago; 2) Medicine, Univ. of Chicago; 3) Pediatrics and Committee of Genetics, Univ. of Chicago; 4) Pediatric Endocrinology, Security Forces Hospitals, Riyadh, Saudi Arabia; 5) Prince Abdullah Hospital, Bisha, Saudi Arabia; 6) Pathology, Adelaide and Meath hospital, Dublin, Ireland.

Incorporation of selenocysteine (Sec) through recoding of the UGA stop codon creates a unique class of proteins. Several factors are required for Sec incorporation: cis-acting sequences present in the mRNA of a selenoprotein (UGA codon and Sec insertion sequence SECIS) and trans-acting factors (elongation factor eEF^{Sec}, $tRNA^{Sec}$ and SECIS-binding protein SBP2). While mice lacking $tRNA^{Sec}$ die in-utero, the in-vivo role of all other components is unknown and no Sec incorporation defects have been described in humans. Deiodinases are selenoproteins responsible for thyroid hormone (TH) metabolism by converting the precursor T_4 into the active (T_3) or inactive (rT_3) hormone. We report two families with abnormal thyroid function tests (high T_4 and rT_3, low T_3 and high TSH) suggestive of abnormal TH metabolism. Family A is of Bedouin origin with 3 of 7 siblings being affected, and family B of Irish origin with one affected child. Administration of incremental doses of T_4 and T_3 indicated that the affected have a defect in generating the active hormone T_3 from T_4. In vitro studies using patients fibroblasts showed decreased deiodinase 2 (DIO2) enzymatic activity but normal DIO2 mRNA expression. Linkage to *DIO2* locus was negative; therefore, we considered a putative post-transcriptional defect altering the availability of active DIO2 enzyme. Systematic linkage analysis with genes involved in DIO2 synthesis and degradation led to the identification of the first inherited Sec incorporation defect, caused by recessive *SBP2* gene mutations: a homozygous mutation in family A and compound heterozygous mutations in family B. Insight into consequences of SBP2 defect is novel and represents an interesting example of an epistatic effect resulting in deficiency of selenoproteins.

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A new defect in -oxidation presenting as sudden liver failure. *M. He[1], S.L. Rutledge[2], D. Kelly[2], E. Goetzman[1], J. Vockley[1].* 1) Deparment of Pediatrics, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 2) Department of Pediatrics, University of Alabama, Birmingham, AL.

The ACADs are a family of at least nine flavoenzymes involved in the mitochondrial -oxidation of fatty acid. Genetic disorders have been identified in seven of the ACADs. ACAD9 is a recently identified ACAD that has maximum activity with unsaturated long chain acyl-CoA substrates. The physiologic role of this enzyme remains unclear, however, as it overlaps the substrate specificity of VLCAD. While both ACAD9 and VLCAD are abundantly expressed in liver, muscle and heart, ACAD9 is highly expressed in the brain, a pattern unique among the other known ACADs. We have now identified the first patient with a deficiency of ACAD9. The patient was a 14 year old apparently healthy boy who died of sudden liver failure following an otherwise mild viral illness. An extensive clinical evaluation was inconclusive but suggested an abnormality of long chain fat metabolism. Acylcarnitine profiling of postmortem liver and skeletal muscle extracts showed elevation of unsaturated long chain species in a pattern that matched the substrate specificity of ACAD9. Autopsy revealed hepatosteatosis and moderate to severe chronic neuronal loss in brain, mirroring the expression profile of ACAD9. Western blotting confirmed absence of ACAD9 in patient fibroblasts and significantly decreased levels in liver and muscle, while northern blotting of mRNA revealed a marked reduction of mRNA of normal size and the presence ACAD9 mRNA of abnormal size. PCR analysis of fibroblast and liver mRNA failed to detect normal ACAD9 species. Instead, multiple mis-spliced species were present, suggesting a defect in splicing. Sequencing of ACAD9 genomic DNA from the patient identified a promotor mutation on one allele that led to dramatically decreased transcription, while several potential pathogenic intron changes near intron-exon boundaries were present on the other allele. The dramatic and sudden nature of this patient's clinical presentation makes it imperative to further characterize of this disorder in individuals with unexplained liver failure and to identify the full spectrum of symptoms.

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MMAHCC is mutated in patients with methylmalonic aciduria and homocystinuria, _cblC_ type. *J.P. Lerner-Ellis[1,3], J.C. Tirone[1,3], P.D. Pawelek[2], C. Doré[4], J. Atkinson[5], T.M. Fujiwara[1,3], D. Watkins[3], C.F. Morel[1,3], E. Moras[1,3], A.R. Hosack[3], C.M. Dobson[6], R.A. Gravel[6], P. Lepage[4], J.W. Coulton[2], J.M. Rommens[5], K. Morgan[1,3], D.S. Rosenblatt[1,3].* 1) Dept. Human Genetics, McGill Univ., Montreal, QC; 2) Dept. Microbiology and Immunology, McGill Univ., Montreal, QC; 3) Div. Medical Genetics, Dept. Medicine, MUHC, Montreal, QC; 4) McGill Univ. and Genome Quebec Innovation Centre, Montreal, QC, Canada; 5) Dept. Molecular and Genomic Biology, Univ. of Toronto, Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 6) Dept. Biochemistry and Molecular Biology, Univ. of Calgary, Calgary, AB, Canada.

Methylmalonic aciduria and homocystinuria, *cblC* type (OMIM 277400) (*cblC*) is the most common inborn error of vitamin B_{12} metabolism with over 250 cases now known. Clinical findings include developmental, hematologic, neurologic, metabolic, ophthalmologic, and dermatologic manifestations. There are at least two distinct phenotypes differentiated by the age of onset. The most severely affected patients present in the first year of life with systemic, neurologic, hematologic, and ophthalmologic findings. Among patients who come to medical attention after the age of 4 years, neurologic symptoms are predominant. We identified the *MMAHCC* gene in chromosome region 1p34.1 and found 17 different mutations in 31 *cblC* patients. A single mutation (c.271dupA, p.R91K-fsX14) accounted for 41% of mutant alleles and, when homozygous, was associated with severe early-onset disease. A nonsense mutation (c.331C>T, R111X) was homozygous in two unrelated patients of Cajun origin, who also had early-onset disease; another nonsense mutation (c.394C>T, R132X) was homozygous in a patient of Pakistani origin, who presented only at 12 years of age. Mutations in *MMAHCC* are consistent with a loss of function of the protein product and several correlate with the age of onset of disease. Molecular modeling predicts that the C-terminal region of the gene product folds similarly to TonB, a bacterial protein involved in energy transduction for vitamin B_{12} uptake. The TonB-like domain may function in protein-protein interactions.

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Biochemical characterization of a murine model of mut class methylmalonic acidemia suggests an important role for skeletal muscle in the production of metabolites. *R.J. Chandler, A. Chen, C.P. Venditti.* Genetic Diseases Research Branch, NHGRI, NIH, Bethesda, MD.

The hereditary of methylmalonic acidemias are a devastating group of autosomal recessive metabolic disorders caused by a defect in the isomerization of L-methylmalonyl-CoA to succinyl-CoA. To study this condition, we have generated two new null alleles at the murine methylmalonyl-CoA mutase (MCM) locus and characterized the biochemical phenotype of the mutant animals. One allele has loxP sites flanking the initiator encoding second exon and is in cis with a deletion of the coenzyme A binding pocket encoded by the third exon. The other allele has the second and third exons of the MCM gene removed and replaced by a single loxP site. The homozygous mutant animals display many features seen in the human condition, including massive elevations of MMA in body fluids and increased production of 2-methylcitrate. Metabolite measurements were made in the prenatal and neonatal periods on brain, liver, kidney, skeletal muscle, urine and plasma. Plasma MMA levels in utero are elevated in affecteds (176uM) versus controls (6uM). Significant increases in the plasma compartment occur over time as the mice become ill and eventually perish but plasma levels never exceed 1.5 mM, even at the end of life. In the first few hours after birth, the MMA content per gram wet-weight is highest in the muscle and kidney. Changes occur near the end of life, with massive elevations in the liver predominating, followed by skeletal muscle, kidney and brain. Murine embryonic fibroblasts derived from homozygous animals show diminished C14-propionate incorporation and can be partly corrected by retroviral transduction with a wild-type murine cDNA. The characterization of the phenotype in this murine model provides insight into the human condition, particularly the persistent MMAemia/uria seen in patients who have received combined liver-kidney transplants and suggests that during homeostasis, the bulk of the plasma MMA in patients derives from the skeletal muscle. A metabolic profile in the mutant animals has been established and will allow future transgenic, viral and enzymatic therapies to be assessed.

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Molecular Analysis of *AASS* in Familial Hyperlysinemia (FH). *N. Braverman[1], A. Lossos[2], U. Vester[3], E. Christensen[4], R.P. Cox[5], M.T. Geraghty[6], N.M. Kim[1].* 1) Johns Hopkins Med Ctr, Baltimore, MD; 2) Hadassah University, Jerusalem, Isreal; 3) Univ. of Essen, Germany; 4) Righospitalet, Copenhagen, Denmark; 5) Univ. Texas Southwestern Med Ctr, Dallas, Tx; 6) CHEO, Ottawa, Canada.

Inborn errors of lysine catabolism represent a rare group of incompletely characterized disorders manifesting with hyperlysinemia, saccharopinuria, aminoadipic aciduria or pipecolic acidemia. One of these, FH, is due to deficiency of the bifunctional enzyme, -aminoadipic semialdehyde synthase (AASS), which catabolizes lysine to saccharopine (lysine ketoglutarate reductase, LKR), and then to -aminoadipic semialdehyde (saccharopine dehydrogenase, SDH) and results in markedly elevated lysine in body fluids.
Less than 50 patients with FH have been described; around half have mental retardation and spasticity. However, molecular analysis has been reported for only one. We report here 9/10 *AASS* mutant alleles in 5 additional probands with FH and psychomotor retardation. These variations were not identified in 100 control genes. Furthermore, we show by RTPCR and sequencing that *AASS* undergoes alternative splicing in several tissues to produce transcripts corresponding to monofunctional SDH and LKR. To determine the effect of the missense changes on AASS activity, we developed an assay in which expression of human AASS and monofunctional SDH rescue the growth phenotype for SDH deficient yeast in lysine free media. In this system, expression of these proteins containing the patient mutations did not rescue. Finally, we localized epitope tagged-AASS and its isoforms to the mitochondria in human cells by indirect immunofluorescence. The relationship between FH and psychomotor retardation is unclear. One of our probands, the product of a consanguineous union, has a similarly affected brother and affected, but asymptomatic parents. We suggest that FH is necessary but not sufficient for neurological disease. The presence of mono- and bifunctional enzymes enables more complex regulation of lysine catabolism that might be important in determining phenotype. Ultimately these investigations will provide better understanding of phenotype determination in FH.

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Secondary creatine deficiency in ornithine delta-aminotransferase deficiency. *K. Mention, N. Boddaert, S. Romano, L. Hubert, J. Kaplan, J.L. Dufier, V. Valayanopoulos, F. Brunelle, J.M. Saudubray, A. Munnich, P. de Lonlay.* Hôpital Necker-Enfants Malades, Paris, Paris, France.

Ornithine delta-aminotransferase (OAT) deficiency is an autosomal recessive condition causing gyrate atrophy of the retina responsible for blindness during the first decade, as a consequence of high plasma ornithine concentrations. Because creatine synthesis requires the reversible conversion of arginine and lysine into ornithine and guanidinoacetate, secondary creatine deficiency was expected to occur in OAT. The aim of this study was to evaluate creatine metabolism in OAT deficient patients and establish correlations with the severity of the neurological involvement. A retrospective study included 7 OAT deficient patients, aged from 12 to 28 years. The median age at diagnosis was 10 years (9-12 yrs). Interestingly, the highest plasma ornithine levels (900mol/l, N<100) were found in children with cognitive and behaviour problems (5/7), with severe mental retardation (4/7, IQ 50-60), leading to special schooling (3/7) and epilepsy (2/7). One patient had hyperactivity and severe behaviour disorder but no mental retardation (IQ=80). Two patients with mean plasma ornithine at 400mol/l had normal neuro-psychological evaluation (IQ = 80) and normal schooling. Cerebral 1H magnetic resonance spectroscopy revealed a striking creatine deficiency in the 4/7 mentally retarded OAT deficient patients and in the patient with severe behaviour disorder but not in the two patients with no mental retardation. These data were confirmed by decreased levels of plasma and urinary creatine and guanidinoacetate (plasma creatine <5mmol/l, N between 6 and 50; urinary creatine 20 mmol/mmol creatinine, N: 17-720; plasma guanidinoacetate 0.5mmol/l, N: 1-3.5; urinary guanidinoacetate <3 mmol/mmol creatinine, N >4). In conclusion, we provide here the evidence of secondary creatine deficiency in OAT deficiency. Moreover we show that the level of creatine deficiency correlated with the severity of mental retardation and hyperornithinemia. The evidence of cerebral creatine deficiency in the disease may be clinically important as creatine administration may improve the clinical course of the disease.

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Expanded Newborn Screening identifies maternal primary carnitine deficiency. *S.A. Berry[1], K. Bentler[1], M. McCann[3], N. Longo[4], M. Pasquali[4], P. Rinaldo[2], D. Matern[2], L.A. Schimmenti[1].* 1) Dept Pediatrics, Univ Minnesota, Minneapolis, MN; 2) Biochemical Genetics Laboratory, Mayo Clinic, Rochester, MN; 3) Minnesota Department of Health, Minneapolis, MN; 4) Dept Pediatrics, Univ Utah, Salt Lake City, Utah.

Expanded newborn screening (NBS) has resulted in tremendous improvement in early identification and treatment of newborns with disorders of fatty acid oxidation and other inborn metabolic errors. An additional benefit of expanded NBS is the identification of previously undiagnosed maternal metabolic conditions. We report the detection of maternal primary carnitine deficiency in two unrelated mothers through expanded NBS in the state of Minnesota. Newborn screening blood spot free carnitine values for two full-term, uncomplicated births were 3.1 uM/L and 2.5 uM/L (abnormal low<7) respectively without elevation of acylcarnitine species. Follow up studies of their asymptomatic mothers showed carnitine depletion, with plasma free carnitine values of 5 uM/L and 2 uM/L (LLN 25 uM/L). Neither mother had a history of hospitalization for Reye-like illness or cardiomyopathy and both had normal echocardiograms. Both infants were treated with low dose oral L-carnitine with rapidly increasing carnitine levels. Both mothers were treated with oral L-carnitine. After 8 months of treatment, one mother has normal plasma free carnitine levels (35 uM/L) on 2 g carnitine/day while after 6 months of treatment the second mother continues to have low plasma free carnitine levels (4 uM/L) despite dosage of 3 grams of carnitine/day. Fibroblast uptake studies show that each mother has defective carnitine uptake, 17% and 3.3% of controls respectively. Mutation analysis of the gene encoding OCTN2 is pending. These cases demonstrate an added value of expanded NBS: diagnosis of unforeseen maternal metabolic disorders.

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Fabry disease: Early clinical manifestations and age at clinical events in a cohort of 1214 males and females. *C.M. Eng[1,2], W.R. Wilcox[2], S. Waldek[2], G. Linhorst[2], D. Germain[2], J. Charrow[2], C.R. Scott[2], F. Breunig[2], M. Banikazemi[2].*
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Fabry disease (FD) is an X-linked lysosomal storage disorder due to the deficiency of a-galactosidase A and accumulation of globotriaosylceramide (GL-3) in visceral tissues and body fluids. The early mortality is caused by end stage involvement of kidney, heart and brain. Enzyme replacement therapy for FD has been shown to clear the vascular endothelial accumulation and to decrease the incidence of significant clinical events in treated patients. Early institution of therapy may prove most effective; however, failure to diagnose affected individuals based on recognition of early symptoms compromises these efforts. The Fabry Registry (www.FabryRegistry.com) was analyzed to determine presenting symptoms, age at diagnosis, and age at clinical events in 1214 individuals with FD (males N=694 and females N=520). The median age of diagnosis was 24 yr for males (N=667) and 34 yr for females (N=476). Males with FD presented with symptoms at a median reported age of 9.0 years (N = 492). Males with FD had initial presenting symptoms most commonly present in the neurologic system (62%) (with 58% responding reporting pain), followed by skin (28%), gastroenterologic (19%), and renal (14%). Females with FD presented with symptoms at a median age of 12.0 years (N=253). At presentation, females had symptoms most commonly present in neurologic (43%), specifically pain (39%), ophthalmologic (13%), gastroenterologic (13%) and skin (11%). Information was collected regarding the age at which a serious complication occurred in a target organ. The mean age (SD) was 39.8 yr (9.8) (N=130 events reported) for dialysis or kidney transplantation, 41.5 (13.3) (N=259) for cardiac events (myocardial infarction, arrhythmia, angina, congestive heart failure or significant cardiac procedure), and 41.3 (11.9) (N=85) for cerebrovascular events (stroke). Awareness of the early course of FD and common presenting signs and symptoms in males and females may lead to improved recognition and outcome for affected individuals.

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Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. *T. Fukuda, K. Zaal, E. Ralston, P. Plotz, N. Raben.* NIAMS/NIH, Bethesda, MD.

The lysosomal storage disorders are a group of more than forty diseases characterized by accumulation of undigested substrate in the lysosomes. In Pompe disease, a deficiency of acid alpha-glucosidase, glycogen accumulates primarily in cardiac and skeletal muscle. Ongoing clinical trials with recombinant human enzyme (ERT) and our pre-clinical studies with the recombinant or transgenic enzyme in KO mice have demonstrated that glycogen is cleared well from cardiac but not skeletal muscle. This modest outcome of ERT highlights the gaps in knowledge of the pathogenesis of the disease and of the downstream pathways affected as a result of lysosomal enlargement. Analysis of the KO myoblasts has shown that the deficiency results in a broad secondary expansion of all vesicles of the lysosomal degradative system. Real-time imaging revealed the overcrowding and stasis in the diseased cells as shown by significantly decreased and disordered movement of late endocytic compartments in KO cells. Similar changes, which may occur in several lysosomal diseases, were found in skeletal muscle from KO, but despite these changes, type I fibers respond better to ERT than type II fibers. We have found that various lysosomal trafficking proteins - CI-MPR, clathrin, TfR, adaptor protein AP-1 and AP-2, and GGA2 - are much less abundant in type II than type I fibers, suggesting that intrinsic properties of different fiber types may contribute to resistance to therapy. But the most striking difference between the fiber types is that only type II fibers from the KO contain enormous regions of autophagic buildup that span the entire length of the fibers. These regions can be seen best by confocal microscopy of isolated single type II fibers. Furthermore, the autophagic vacuoles remained abundant in ERT-treated type II fibers. We suggest that the role of the enzyme in skeletal muscle is broader than previously thought, and that the pathogenesis of the disease is different in the two fiber types. The vastly increased regions of autophagy, a process known to be induced by starvation, may cause skeletal muscle destruction and prevent efficient delivery of the therapeutic enzyme.

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Is a genetic questionnaire as accurate as pedigree analysis in the evaluation of genetic infertility and risks to offspring? *K.L. Danziger[1], M. Brown[1], L.D. Black[2], P.J. Turek[1].* 1) University of California, San Francisco, San Francisco, CA; 2) California Pacific Medical Center, San Francisco, CA.

Introduction and Objective: For couples seeking fertility care, a comprehensive family history assessment can add valuable information about genetic etiology and possible risks for offspring. To elicit personal and family history information, we created a genetic questionnaire and asked whether it could capture information similar to pedigree analysis. Here, we report our findings on comparison of these two instruments. Methods: A consecutive series of infertility patients completed a genetic questionnaire. These patients also had a formal genetic pedigree obtained by a genetic counselor. We compared the ability of these 2 instruments to elicit information concerning 5 areas of personal and family medical history: recurrent miscarriages, stillborn births, developmental delay/learning disabilities/mental retardation, heart defects, and birth defects. The pedigree was considered the gold standard and the genetic questionnaire scored against it for accuracy. Results: Among 104 completed questionnaires and pedigrees, 76 (73 %) patients indicated relevant genetic information in one of the 5 key areas. A comparison of all findings elicited by the two methods revealed that 61 (80%) patients failed to report key genetic information that was ascertained by formal pedigree analysis. Specifically, the questionnaire missed 21/28 (75%) occurrences of stillborn births, 21/24 (88%) cases of birth defects, 14/20 (70%) cases of developmental delay/learning disabilities/mental retardation, 12/14 (86%) cases of miscarriages, and 11/13 (85%) cases of congenital heart defects. Conclusions: Family history information may provide valuable information to infertile couples about the causes and implications of their infertility. As this study shows, accuracy of family history information depends on ascertainment method. A comprehensive family medical history questionnaire is not as reliable for capturing relevant, genetic information as a pedigree analysis. The optimal method to ascertain such information will become more important as our knowledge of genetic infertility and its implications expands.

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ALTERNATIVES TO TRADITIONAL GENETIC COUNSELING: USE OF AN EDUCATIONAL PRECOUNSELING CD-ROM. *S. Bhatt[1], T. Cousineau[2], D. Cutillo[1], A. Cronister[1].* 1) Genzyme Genetics, Orange, CA; 2) Inflexxion, Newton, MA.

Traditional prenatal counseling involves meeting with a genetic counselor to review the referral indication and discuss appropriate testing based on individual risk assessment. With the expanding knowledge about carrier testing, genetic counselors find themselves spending more time reviewing information unrelated to the referral indication. For patients, this results in decreased satisfaction and value of the counseling session. As a precounseling tool, an educational CD was developed to address repetitive information such as carrier testing, serum screening and diagnostic options. Two patient surveys were conducted, one by patients who had genetic counseling only, and a second by patients who viewed the precounseling CD followed by genetic counseling. Both surveys focused on patients' understanding of and decisions about genetic testing. The CD survey also asked about the ease of use and length of the CD. Results were compared to determine the usefulness of the CD. 202 patients were surveyed from four clinics in three states. 56 patients completed the GC only survey and 146 patients completed the CD survey. 97% found the CD easy to use, and 93% said it adequately addressed genetic testing information. In addition, 97% in the CD survey group indicated a good to excellent understanding of available testing, which was no different than the GC only group. The main difference noted was in the area of patient decision making. Overall and prior to counseling, about 30% of patients indicated they had already made up their mind about genetic testing; 20%; were willing to reconsider their decision and 50% had not begun, were in the process or would make a decision after learning about options. Although 40% of patients said that the CD helped them to make decision regarding genetic testing, a greater number of patients (63%)said that the genetic counseling further influenced their decision about genetic testing. Our survey indicates that the CD is easy to use and provides an excellent adjunct to the genetic counseling session. Genetic counseling continues to provide a setting for discussion of patients' specific issues and making decisions regarding testing.

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Recontacting research participants: Implications for genetic banking studies. *M.E. Smith, S.A. Aufox, R.L. Chisholm, W.A. Wolf.* Ctr Genetic Medicine, Northwestern Univ, Chicago, IL.

NUgene is an IRB approved gene bank, established in 2002 at Northwestern University. Participants are recruited from a diverse population receiving care through a Northwestern University healthcare affiliate. During the consenting process, participants agree to the broad use of their DNA sample in genetic research. Initial IRB approval of the project was granted on the condition the consent form allow participants to indicate their preference to be contacted in the event that medically significant results were found during the conduct of research. The IRB was asked to reconsider this option based on ethical concerns and in November 2004 granted the request and the option was removed. An IRB approved letter and consent form addendum, allowing participants to indicate their preference about remaining in the study, were mailed to 1647 participants who had agreed to this option. Participants were informed they could indicate their decision about remaining in the study by mail, phone, or through a secure website. Between February and April 2005, letters were sent using the contact information provided during the time of enrollment. Eighty letters (4.9%) were returned as undeliverable. As of June 1, 2005, 689 individuals responded(41.8%), 551(80%) agreed to continue participation in NUgene. Of the 97(14%) declining to continue participation, the majority indicated the change in study protocol as their main reason for withdrawing. Few reports in the literature describe experiences recontacting participants in a large DNA bank. Most respondents appeared to understand the initial consent form and were willing to participate regardless of receiving personal results. Contacting and reconsenting research participants was challenging even though participants provided information for this purpose. Some researchers and ethicists have proposed that participants in DNA banking projects, like NUgene, should be consented for each study using their sample. These results may have implications for the use of blanket consent forms in genetic research and the difficulty in recontacting and obtaining consent for specific studies.

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Direct contact, without considering the index patient, to offer information and counseling to HNPCC-families. *H. Kääriäinen[1], K. Aktan-Collan[2], K. Pylvänäinen[3], J-P. Mecklin[3].* 1) Department of Medical Genetics, University of Turku, Turku, Finland; 2) Department of Clinical Genetics, Hospital District of Helsinki and Uusimaa, Helsinki, Finland; 3) Department of Surgery, Jyväskylä Central Hospital, Jyväskylä, Finland.

In familial cancer syndromes, the relatives are usually offered genetic counseling, follow up examinations and/or genetic testing via the index counselees (cascade screening). In many families, however, there are branches that cannot be approached by this method. The purpose of our study was to evaluate the consequences of direct contacting of relatives in HNPCC-families without help or even consent from the index counselees.

We approached 247 high risk HNPCC-family members by a letter explaining that we do research in hereditary cancer and would like to have their written consent to receive a phone call from our research assistant (KP). After the phone call, 144 (46%) accepted to come to genetic counseling and fill in pre- and post-counseling/post- testing questionnaires. Those accepting were younger, male, and married or co-habiting more often than their counterparts. In the counseling, we did not reveal any health data of the relatives but just stated that we knew that there was HNPPC in the family and that we believed that the individual concerned had a 50% risk of having inherited the mutation. Of those accepting, 97% knew that a near relative had cancer, 3/4 felt that there was more cancer than usual in their family and 9/10 thought that directly contacting them had been a good thing.

This positive attitude encourages us to suggest that in cancer families direct contact to the relatives, without considering the index counselees opinion, is acceptable. It has even been stated that, in agreement with the paradigm that genetic information can be considered to be the property of the family, the professionals have a moral duty to try to inform relatives if the proband is unwilling to do so (De Wert: Whose information is it anyway? Eur J Hum Genet 2005:13:397-398).

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The trouble with phenocopies: are those testing negative for a family BRCA1/2 mutation really at population risk? *D.GR. Evans[1], A. Smith[1], MC. Boyd[1], M. Bulman[1], E. Woodward[2], L. Smith[2], A. Moran[1], F. Lalloo[1], E.R. Maher[2].* 1) Genetics, St Mary's Hospital, Manchester UK, United Kingdom; 2) Dept of Human Genetics, Birmingham womens Hospital, Birmingham UK.

Background: The identification of BRCA1 and BRCA2 mutations in familial breast cancer kindreds allows the option of genetic testing of at risk relatives Those who test negative are usually reassured and extra breast cancer surveillance discontinued. However, we postulated that in high-risk families such as those seen in clinical genetics centres, the risk of breast cancer might be influenced not only by the BRCA1/BRCA2 mutation but also by modifier genes. One manifestation of this would be the presence of phenocopies (ie. individuals with breast cancer but without the mutation) within BRCA1/BRCA2 kindreds. Methods: We reviewed 277 families with pathogenic BRCA1/2 mutations and identified 28 breast cancer phenocopies. We assessed the relative risk of breast cancer in those testing negative using incidence rates from our local population based cancer registry. Results: Phenocopies constituted 16% of tests on women with breast cancer following the identification of the mutation in the proband. The standardised incidence ratio for breast cancer for women who tested negative for the BRCA1/BRCA2 family mutation was 5.3 for all relatives, 5.0 for all first-degree relatives (FDRs) and 3.2 (95% confidence interval: 2.0-4.9) for FDRs in whose family all cases of breast and ovarian cancer could be explained by the identified mutation. 13/107 (12.1%) of FDRs with breast cancer and no unexplained family history tested negative. Conclusion: In high risk families, women who test negative for the familial BRCA1/BRCA2 mutation had an increased risk of breast cancer consistent with genetic modifiers. In the light of this such women should be offered continued surveillance.

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Different causes and outcomes of splicing defects in *PTEN*. *S. Agrawal, R. Pilarski, C. Eng.* Human Cancer Genetics, Ohio State University, Columbus, OH.

PTEN, encoding a dual phosphatase tumor suppressor, is mutated in 85% and 65% of individuals with classic Cowden Syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS), respectively. Approximately 23 germline mutations in putative splice sites have been published, but resulting downstream outcome data are limited. We sought to systematically determine splicing defects in *PTEN* in 40 germline PTEN mutation positive and 33 mutation negative cases with classic CS, BRRS and CS- or BRRS-like features, in the first instance. Altered splicing was observed in 4/40 mutation positive probands and 2/33 mutation negative probands. Two splice site mutations (IVS 3+1GA, IVS 3+5 GA) led to the splicing out of exon 3, (IVS 3-1 GA) led to exon 4 skipping and another (IVS 6+1 GT) deleted exon 6. Interestingly, 2 mutation negative BRRS patients skipped exon 3, and later genomic sequencing revealed a mutation (IVS 2-38 insG) in what could be a conserved branch site (UGUUAAU) in intron 2. We then sought to characterize the transcriptional and biochemical outcome of these 5 distinct splice site mutations, which led to the skipping of exon 3, 4 or 6. The splice site mutations leading to deletions of exon 3, 4 or 6 resulted in reduced lipid and protein phosphatase activities of PTEN. Deletion of exon 4 was associated with severely reduced lipid phosphatase activity, whereas the samples with exon 3 skipping had markedly reduced protein phosphatase activity. Furthermore, exon 3 deleted transcript and protein were stable and localized to the nucleus more efficiently than the wild-type PTEN. In contrast, exon 4 skipping resulted in unstable transcripts and severely truncated unstable PTEN protein lacking its phosphatase domain. We have therefore not only described for the first time, the effect of a deep intronic/branch site mutation on exon skipping in *PTEN*, but also found that different splice site mutations resulting in deletion of different exons lead to distinct outcomes.

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Patient Attitudes Towards Follow-Up Letters Provided After Genetic Counseling for Inherited Cancer Risks. *E. Rosenthal, L. Madlensky.* Moores UCSD Cancer Ctr, La Jolla, CA.

Many cancer genetics clinics provide patients with detailed follow-up (F/U) letters summarizing the content of clinic visits. These are distinct from clinic notes used to communicate with other health care providers. Preparation of these letters is time-consuming, but very little systematic research has documented how many patients perceive these letters as useful for themselves and other family members, what content is most valued, the extent to which letters improve understanding and compliance with recommendations, and which formats are most effective. To begin addressing some of these issues we surveyed patients who had received a detailed F/U letter following genetic counseling for hereditary cancer risk at the UCSD Cancer Center between 04/01/01 and 01/30/04. Survey questions addressed demographics, patients assessment of their letters clarity, comprehensiveness, accuracy and usefulness, and attitudes towards the use of the letter as a mechanism for disseminating information within families. Of 220 invited participants, 130 (60%) completed surveys. Most participants were highly educated, Caucasian, and affluent. 80% indicated that they actively seek out health information. Overall, 94% reported reading their letter carefully, with 65% reading it more than once. 80% had retained a copy. Patients expressed high levels of satisfaction with all aspects of the letters. The majority (85 to 95%) characterized the letters as clear, comprehensive, and an accurate summary of their genetic counseling sessions. The greatest expression of dissatisfaction was that 20% would have liked more medical information. A high proportion of patients (79 to 98%) felt the letter would be a useful way to share information with varioius relatives, but a much lower percentage (10 to 33%) reported that they had actually shared their letter. The results of this exploratory study show that these patients appreciate and are satisfied with F/U letters. Further research is needed to explore other formats of providing summaries of the complex information discussed in genetic counseling sessions, particularly in more diverse patient populations.

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The Genetic Discrimination Project: benefit, disadvantage and negative treatment associated with genetic information in an Australian sample of clinical genetic service clients - Australian and international implications.

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Genetic discrimination is defined as differential treatment, either positive or negative, of asymptomatic individuals on the basis of real or assumed genetic status. The Genetic Discrimination Project (GDP) has investigated the experiences of a sample of Australian adults who presented at 15 clinical services regarding predictive genetic testing for various conditions between 1998 and 2003. Of 2362 individuals who received a questionnaire, 1185 (50.2 percent) responded. This paper reports findings relating to respondents perceptions and experiences of benefit, disadvantage and negative treatment associated with having genetic information. On scales measuring perceived overall benefit and disadvantage, 94 percent (905/959) of respondents gave scores indicating some to great benefit and 47 percent (438/932) gave scores indicating some to great disadvantage. Variations occurred across conditions and perceptions of benefit and disadvantage were significantly associated with test result. Specific incidents of alleged negative treatment or discrimination against asymptomatic individuals involving life insurance products,employment, health professionals and family relations were reported by 7.3 percent (87/1185) of respondents. Data contextualising perceived benefits and disadvantage, incidents, responses to them by the consumers and third parties and perceived barriers to making complaints are presented. These 87 accounts comprise part of a total of around 100 accounts of alleged discrimination and negative treatment within this first empirical study that will inform public policy both in Australia and internationally.

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Physician Knowledge and Opinions on Genetic Discrimination. *K. Lowstuter[1], S. Sand[1], C. Lee[2], B. Schwerin[3], G. Uman[4], K. Blazer[1], J. Weitzel[1].* 1) City of Hope Cancer Center, Duarte, CA; 2) California Medical Assoc Foundation, Sacramento, CA; 3) Cancer Legal Resource Ctr, Los Angeles, CA; 4) Vital Research, LLC, Los Angeles, CA.

There is concern that many physicians have inadequate knowledge of genetic discrimination or protective legislation. Perceptions about genetic discrimination may limit access to genetic testing and consequently to risk-appropriate cancer screening and prevention. To explore physician knowledge and opinions on genetic discrimination law, and the extent to which these influence referral practices, we are surveying a representative sample of the 35,000 California Medical Association (CMA) member physicians. **Methods** The survey contains demographic items and 37 items on knowledge, practice, and opinions regarding genetic testing for cancer predisposition. Invitation letters and pre-qualification postcards were sent to a stratified random sample of 1601 CMA members. The survey is available online or on paper. Interval reminders were sent to non-responders using a modified Dillman method (Dillman et al 1974). **Preliminary Results** Of the 226 responders to date (target N=400), 85% chose to complete the online version of the survey rather than paper (p0.001). More agreed with the statement that genetic testing creates health insurance problems for patients without cancer (54%)than for those with cancer (44%) p=0.038. The majority (64%) did not know that federal law prohibits health insurance discrimination in the group market on the basis of genetic information. Of the 83 respondents (37%) who have never referred patients for genetic cancer risk assessment, 17% indicated concern over health insurance discrimination as the reason and 43% stated the referral process was unclear. **Conclusions** Consistent with our previous observations in a population of clinicians attending CME activities at our cancer center (Nedelcu et al 2004), results to date indicate gaps in knowledge and misperceptions that may lead to barriers to referral for cancer risk assessment. Comprehensive and well-publicized protective federal legislation along with direct physician education may help promote access to appropriate care.

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Whose Genetic Profile Is It Anyway? Challenges in Forensic Investigations. *A. Chidambaram, M. Collins, C. Duda, J. Cohen, J. Schuerman, H. Hamilton, C. Beheim.* Alaska State Crime Detection Laboratory, Anchorage, AK.

The need to establish and enforce guidelines for appropriate use of genetic information for medical or research purposes has been widely acknowledged. Varying degrees of state specific regulations currently exist in this country to protect people's right to confidentiality pertaining to their genetic make-up. The US Senate has unanimously voted to pass the Genetic Information Nondiscrimination Act of 2003 (S. 1053). This may adequately address genetic privacy issues regarding equal access to health insurance and employment decisions if the ownership of the genetic information is not in question. However, in the case of identical twins, where 2 individuals have identical genetic profiles, one twin's forfeiture of the right to privacy, voluntarily or involuntarily, could affect the other twin who might be disinclined to compromise his or her genetic privacy. Genetic analysis in forensic investigations can pose ethical dilemmas when defining or determining what constitutes ownership of a genetic profile. Database entry of a convicted (twin) felon's genetic profile automatically enters that of the other twin as well. Requesting fingerprint data to distinguish between the twins could also be viewed as violation of privacy rights. Ethical and analytical dilemmas also arise when one person has 2 distinct genetic profiles. If identified as a suspect in an investigation, a bone marrow transplant recipient's medical history may be subject to disclosure to enable collection of reference sample(s) from appropriate biological tissue(s) to make meaningful genetic comparisons with other forensic sample data. The transplant recipient as well as the donor's right to medical and genetic privacy is in jeopardy here. Civil litigation such as paternity disputes can also be adversely affected by such circumstances. Informed consent policy protocols and education regarding the health, legal / forensic as well as social / ethical implications of undergoing certain medical procedures are necessary as medical technology and human identification methodologies continue to outpace and challenge legal and social expectations of an individual's right and priviledge to privacy.

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A targeted chromosomal microarray for CGH-based analysis of clinically relevant chromosomal disorders and subtelomeric regions. *S. Cheung, C. Shaw, J. Li, Z. Ou, A. Patel, E. Brundage, S. Yatsenko, L. Cooper, S. Trilochan, P. Ward, P. Stankiewicz, W. Cai, J. Lupski, C. Chinault, A. Beaudet.* . Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX.

A CGH-based microarray was developed for clinical diagnosis of well-characterized chromosomal disorders while minimizing detection of variations of uncertain clinical significance. This array contains 366 FISH-verified clones that span genome regions implicated in over 55 known human genomic disorders. The array has multi-clone replication for each disease, with an average of 3-4 clones representing each deletion or duplication syndrome, and 4 or more clones for 41 subtelomeric regions to integrate information and to enhance the specificity and sensitivity of our findings. To accomplish the data integration, we developed custom analysis software in R open source statistical computing language. Our system is also integrated with a relational database so that historical results can be mined to identify polymorphic clones and their genomic contexts. To date, we have completed 728 analyses in which chromosome tests were previously reported normal or performed simultaneously. We identified 1) clinically relevant and well characterized disorders in 47 cases, and 2) deletion and reciprocal duplications of a region in 15q11.2 that includes the breakpoint 1 region for Prader-Willi syndrome (8 cases), the NF1 region in 17q11.2 (2 cases), and the STS and SHOX regions in Xp22.32 and Xp22.33, respectively (4 each). We also detected other single clone abnormalities leading to discovery of genomic imbalances of the nearby region. The major advantages of this selected human genome array include: interrogation of clinically relevant genomic regions, the ability to test for a wide range of duplication and deletion syndromes in a single analysis, and ease of confirmation of suspected genomic changes by conventional FISH tests currently available in the cytogenetics laboratories. Although not intended to substitute for a standard karyotype, this array is an attractive alternative to telomere and locus-specific FISH analysis. Additional results from an updated V.5 array with 860 clones will also be presented.

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Identification of cytogenetic abnormalities by microarray analysis: a study of 1,300 consecutive clinical cases.
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Array-based comparative genomic hybridization (CGH) allows for the simultaneous interrogation of hundreds or thousands of discrete loci for the detection of unbalanced chromosomal constitutions. We present the results of the largest study to date of 1,300 consecutive clinical cases submitted to our diagnostic laboratory for array CGH. Clinical geneticists submitted most cases, with a smaller proportion submitted by neurologists, developmental specialists, and pediatricians. Our laboratory uses the SignatureChip^{TM}, which was designed to detect the common microdeletions, reciprocal microduplications, subtelomeric and pericentromeric alterations, unbalanced translocations, and aneuploidy. The design also allows for distinguishing the common-sized microdeletions that are flanked by low-copy repeats, from larger deletions, through the use of flanking control loci. The array utilizes 831 BACs covering 126 clinical and 104 control loci in 3-6 clone contigs. If the case was known to us to have a chromosome abnormality submitted for clarification, that case was not included in this report. Of the 1,300 clinical cases submitted to our laboratory for diagnostic testing, most had previous or concurrent cytogenetic analysis, subtelomere FISH, and/or locus-specific FISH. We identified DNA copy number alterations in 108 (8.3%) cases. Of these, 22 (1.7%) represent population variants; in 13 (1.0%) cases, the clinical significance is unclear because parental samples were unavailable for testing; and in 73 cases (5.6%) clinically relevant abnormalities were identified. In this latter group, 22 telomeric deletions or unbalanced rearrangements were identified. Interestingly, 21 cases showed syndromic deletions. Our data demonstrate that targeted array CGH detects a substantial proportion of abnormalities even in patients who have already had extensive cytogenetic/FISH testing. Further use of this approach will expand the phenotypes of recognized cytogenetic disorders, uncover the chromosomal etiology of known syndromes, and identify new syndromes.

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Array-MAPH: A novel microarray technology for genomic analysis. *A. Kurg[1], K. Männik[1], L. Kousoulidou[2], C. Sismani[2], C. Pitta[2], D. Andreou[2], O. Zilina[1], S. Parkel[1], C. Tryphonos[2], C. Antoniadou[2], N. Tõnisson[1], P. Palta[1], T. Möls[1], M. Remm[1], P.C. Patsalis[2].* 1) Inst. of Molecular & Cell Biol, University of Tartu, Tartu, Estonia; 2) Dept. of Cytogenetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

The development of accurate and sensitive genome-wide screening methods to detect small genomic imbalances has been a technical challenge for a long time. The focus of this study was to develop a new array-based technology using Multiplex Amplifiable Probe Hybridization (MAPH) principle that will enable the fast, accurate and reliable determination of copy-number changes in any loci of complex genomes. We have developed methodology and software for designing of PCR-amplifiable hybridization probes (200-600 bp) that can be used for microarray MAPH and Comparative Genomic Hybridization (CGH). Probe uniqueness is achieved using MegaBLAST and SSAHA programs and the uniqueness of PCR primers using the GenomeMasker package. We have designed 560 probes for human chromosome X, which cover it uniformly with median distance of 250 kb, while in certain regions of interest with resolution up to 3 kb. Additional 107 probes from the autosomal chromosomes were selected and used as normalization controls. Candidate probes were amplified by PCR with success rate of 95%, and 80% of probes worked with 95% call rate on array. For validation of array-MAPH, we used patient samples with known chromosome X aberrations in the blind studies. Array-MAPH determined exactly the same mutations, showing that the new method is equally accurate and sensitive as array-CGH. Additional FISH and STS analysis precisely confirmed all of the above genomic imbalances found by array-MAPH. The new array-MAPH method offers an alternative methodology to array-CGH and further provides several advantages for high-throughput diagnostic screening and research applications by enabling high specificity and sensitivity as well as a high flexibility to study virtually any region in the human genome. Furthermore, it allows extremely high resolution of genomic analysis by detecting known small size deletions and large-scale copy-number variations of genes or exons.

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Novel Approaches to the Analysis and Detection of Chromosomal Abnormalities: 22q as a Model. *B.S. Emanuel[1], J.A. Vorstman[1], G.R. Jalali[1], A.E. Urban[2], S.M. Weissman[2], M. Snyder[2], NimbleGen Inc..* 1) Children's Hosp of Philadelphia, Philadelphia, PA; 2) Yale University School of Medicine, New Haven, CT.

Non-random, constitutional abnormalities of 22q include translocations and deletions associated with DGS/VCFS, duplications associated with the supernumerary bisatellited marker chromosome of CES and translocations that give rise to the recurrent t(11;22) malsegregation-derived +der(22) syndrome. Cytogenetics and FISH are the methods of diagnosis. The positions of the rearrangement breakpoints have been localized in a large patient group. Most breakpoints cluster in 22q low copy repeats. Deletions span a typically deleted region (TDR) of ~3 Mb in >85% of patients. There are recurrent variant breakpoints and atypical deletions. Further, there are patients with the del(22) phenotype in whom deletions have not been detected and some in whom duplications of the TDR have been detected. The frequency of abnormalities of 22q makes it an attractive model for developing more sensitive diagnostic assays. Toward this end, we have utilized a multiplex ligation dependent probe amplification (MLPA) kit (MRC-Holland, Amsterdam) and oligonucleotide-based array CGH to assess DNA copy number. Both methodologies have been used on a training set of known samples containing one, two, three and four copies of 22q DNA sequence as well as unknown 22q abnormalities. We have shown that MLPA provides a rapid, sensitive, quantitative multiplex PCR approach to copy number determination as an initial screening method. Using a maskless array synthesis platform, in situ synthesis of 393,000 oligonucleotide probes has been performed on a single microarray. Probes are spaced ~85 bp apart (repeat sequences are masked) tiling through both strands (sense and antisense) of 22q. An isothermal format is employed where probe lengths range from 45 to 85 nt (Tm = 76C), resulting in more consistent probe performance. This high-density approach enabled ultra-high resolution mapping of DNA copy number and successfully identified all abnormalities detected by FISH and MLPA with exquisite precision. Thus, oligonucleotide-based aCGH is emerging as a powerful method for investigating changes of chromosomal structure.

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Interstitial deletion of chromosome 9q22.32-q22.33 : a novel cause of syndromic overgrowth. *R. Redon[1], G. Baujat[2], D. Sanlaville[2], M. Le Merrer[2], M. Vekemans[2], N. Carter[1], A. Munnich[2], L. Colleaux[2], V. Cormier-Daire[2].* 1) The Wellcome Trust Sanger Inst, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom; 2) Department of Medical Genetics and INSERM U393, Hôpital Necker Enfants Malades, Paris, France.

Overgrowth syndromes are characterized by a height over<+>2 SD in association with a variable combination of macrocephaly, mental retardation, facial dysmorphic features, advanced bone age, and hemihyperplasia. Apart from well know conditions and chromosome anomalies - such as Sotos syndrome or del(22)(q13.1q13.2) - a number of overgrowth patients remain undiagnosed. In the course of a systematic screening using array-based Comparative Genomic Hybridization (array-CGH) of patients with unexplained overgrowth syndrome, we identified two children with congenital overgrowth and nearly identical de novo interstitial deletions del(9)(q22.32-q22.33). The clinical manifestations include macrocephaly, pre and postnatal overgrowth (<+>2.5 SD), and distinctive facial features (frontal bossing, down-slanted palpebral fissures, epicanthal folds, long tubular nose, small triangular mouth with thin upper lip, long philtrum, low-set ears). In addition, both children present with psychomotor delay, hyperactivity and strabismus. Further analysis with a custom microarray covering the whole breakpoint intervals with fosmids, allowed to delineate the deletion breakpoints within 100-kb intervals and demonstrated that the deletion boundaries, although close, are different for the two patients. Interestingly, the common deleted region encompasses the TGFBR1 gene, a gene which mutations are responsible for craniofacial, neurocognitive and skeletal development anomalies. We suggest therefore that the 9q22.32-q22.33 micro-deletion syndrome is a novel cause of overgrowth and mental retardation with distinctive facial features. Ongoing studies on TG signalling will provide further evidence of the involvement of TGFBR1 in the pathogenesis of this novel phenotype.

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Cleidocranial Dysplasia Plus Vascular Anomalies With 6p21.2 Microdeletion Spanning RUNX2 and VEGF. *K. Izumi[1], N. Yahagi[2], Y. Fujii[2], R. Kosaki[3], M. Higuchi[4], Y. Naito[1], G. Nishimura[4], T. Takahashi[1], K. Kosaki[1].* 1) Dept Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept of Pediatrics, Yokohama Municipal Citizen's Hospital, Kanagawa, Japan; 3) Dept of Clin Genet & Mol Med, Nat'l Children's Med Ctr, Tokyo, Japan; 4) Dept of Radiol, Tokyo Metropolitan Kiyose Children's Hosp, Tokyo, Japan.

Vascular endothelial growth factor (VEGF) is an endothelial-cell-specific mitogen and angiogenic factor, as well as a potent mediator of vascular permeability. Recently, a critical role of Vegf in embryonic vasculogenesis was documented in mice. Loss of Vegf function (Vegf164 isoform) led to abnormal pharyngeal arch development, reminiscent of the condition seen in del22q11 patients. In humans, a genetic epidemiology study revealed that a VEGF polymorphism shown to reduce VEGF expression in vitro was associated with an increased risk for cardiovascular defects in del22q11 individuals. Based on the mouse mutant model and human epidemiological data, researchers hypothesized that a human VEGF mutation would lead to congenital malformations of arterial patterning in the pharyngeal arch arteries. However, no patients with germline mutations in VEGF have been reported to date. We here describe a patient with cleidocranial dysplasia (CCD) who had the classic features such as large fontanelles and hypoplasia of the clavicles plus an aberrant patterning in the pharyngeal arch arteries, including a vascular ring around the trachea and esophagus and an absent left internal carotid artery, and developmental delay. FISH analyses revealed a microdeletion of chromosome 6p21 encompassing the RUNX2 and VEGF loci. An array CGH analysis revealed an interstitial deletion of the short arm of chromosome 6, as indicated by hemizygosity of three BAC (RP3-447E21, RP11-79F13, and RP11-121G20) spanning 1.8 megabases. To our knowledge, the patient described in this report is the first patient with a germline mutation of the VEGF locus. The similarity between the vascular phenotype of the present case and that of Vegf-deficient mice strains suggests that the abnormal arterial patterning in the present case may be attributed to hemizygosity at the VEGF locus.

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ZNF674: A NOVEL XLMR GENE IDENTIFIED THROUGH ARRAY CGH. *A.P.M. de Brouwer[1], D. Lugtenberg[1], M. Banning[1], A. Oudakker[1], H.V. Firth[2], L. Willatt[2], J. Chelly[3], J.P. Fryns[3], H.H. Ropers[3], C. Moraine[3], H.G. Brunner[1], H.G. Yntema[1], H. van Bokhoven[1].* 1) Department of Human Genetics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Department of Medical Genetics, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; 3) European XLMR consortium: www.EURO-MRX.com.

Array-based comparative genomic hybridization (array CGH) is a promising new technique that allows the detection of submicroscopic chromosomal aberrations that cannot be discovered by traditional cytogenetic methods. We analyzed a boy with a possible X-linked contiguous gene deletion syndrome by array CGH. Hybridization of full coverage X-chromosomal BAC arrays revealed a deletion of approximately 1 Mb in Xp11.3 harboring RP2, SLC9A7, CHST7 and two hypothetical zinc finger genes ZNF673 and ZNF674. Partial deletion of the RP2 gene is very likely responsible for the retinal degeneration in the patient. The other genes were considered candidates for the XLMR and were screened for mutations in 28 non-specific XLMR families with an overlapping linkage interval at Xp11.3. One nonsense mutation, p.E171X, was identified in ZNF674. Therefore, another 306 XLMR patients without a linkage interval were tested for mutations in this new KRAB-containing zinc finger (KRAB/ZNF) gene. Two more nucleotide changes, p.P464L and p.M395T, were identified. These three changes were not present in 350 control X-chromosomes. The nonsense mutation p.E171X leads to a truncated protein that has no zinc finger domains. The Pro to Leu change is present in a conserved linker between two zinc finger domains and probably causes a disturbance of its DNA binding property. The second missense mutation, p.M395T, is located between two zinc fingers but involves an amino acid that is not conserved in other zinc finger genes. Besides ZNF41 and ZNF81, ZNF674 is the third gene in the Xp11 KRAB/ZNF gene cluster that is mutated in XLMR patients. Interestingly, this KRAB/ZNF gene cluster on Xp11 seems to be specific for primates. These results show that ZNF674 plays a role in XLMR and is important for cognitive functioning in humans.

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High-resolution analysis of genomic imbalance in children with mental retardation. *J.M. Friedman[1], L. Arbour[1], L. Armstrong[1], D. Bailey[3], A. Baross[4], P. Birch[1], M. Brown-John[4], M. Cao[3], S. Chan[4], D.L. Charest[4], A. Delaney[4], P. Eydoux[2], N. Farnoud[4], N. Fernandes[1], S. Flibotte[4], W. Gibson[1], A. Go[4], R. Hanson[4], R. Holt[4], S. Jones[4].* 1) Dept. of Medical Genetics, U. of British Columbia, Vancouver, Canada; 2) Dept. of Pathology and Laboratory Medicine, U. of British Columbia, Vancouver, Canada; 3) Affymetrix, Inc., Santa Clara, CA; 4) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, Canada.

The most frequent recognized cause of mental retardation (MR) is gain or loss of genomic material. Some pathogenic genomic copy number variants (CNVs) can be identified by conventional cytogenetic studies, but most require molecular cytogenetic techniques for detection. Submicroscopic CNVs are also frequent in normal individuals, and these normal variants must be distinguished from the pathological genomic imbalances that cause MR.

We are studying constitutional CNVs in 100 trios comprised of a child with idiopathic MR and both clinically normal parents using genomic arrays with 50-100 kb resolution. De novo CNVs identified in an affected child but absent in both parents are presumed to be potentially pathogenic, while CNVs found in at least one parent are presumed to be polymorphisms unrelated to the childs MR. Whole-Genome Sampling Analysis using Affymetrix 100K GeneChip Mapping Arrays with Affymetrix CNAT, dChip or custom segmentation analysis software reveals apparent CNVs in these children and their normal parents. Some of the de novo changes detected in the children with MR have been confirmed by locus-specific FISH and are likely to be pathogenic, while other apparent changes cannot be confirmed by independent techniques and probably reflect limitations of the technology or our current approaches to bioinformatic analysis. The samples are also being studied by comparative genomic hybridization on our 32K BAC arrays (Krzywinski et al. Nucleic Acids Res. 2004; 32:3651) and on a whole-genome oligonucleotide platform to permit direct comparisons of these high-resolution technologies and the software for detection of pathogenic genomic CNVs in children with MR.

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Rapid prenatal diagnosis by microarray-based comparative genomic hybridization: Experience of a pilot program. *T. Sahoo, A. Patel, P. Ward, S. Darilek, J. LI, D. DelGaudio, S. Kang, S. Lalani, I. Van den Veyver, S. McAdoo, A. Burke, B. Roa, C. Shaw, C. Chinault, S. Cheung, A. Beaudet, C. Eng.* Molec & Hum Gen, Baylor Col Medicine, Houston, TX.

Microarray-based comparative genomic hybridization (a-CGH) is a new tool for the identification of chromosomal aberrations. We conducted a study in the prenatal setting to evaluate 1) reliability of a-CGH to detect abnormalities in amniocentesis and CVS samples, 2) a program to provide patient education and support, and 3) acceptance of a-CGH as a prenatal test when offered at no additional cost. Our microarray included 366 genomic clones covering all sub-telomeric regions and loci implicated in over 55 genetic disorders. A counseling protocol and educational materials addressing the risks and benefits of a-CGH were discussed during the informed consent process. For the 77 couples participating (44 AF, 32 CVS and 1 fetal blood), indications included advanced maternal age (n=50), fetal ultrasound anomalies (n=15), previous history of affected child (n=9), and abnormal serum screening (n=3). For 35 samples, DNA extracted directly from uncultured amniocytes or CVS samples gave successful a-CGH analysis often with a whole-genome-amplification step, permitting rapid result reporting. There was 100% concordance between the standard karyotype and a-CGH including 4 cases of trisomy 21. In 12 cases, initial a-CGH analysis detected a gain or loss requiring study of parental samples; a familial variant was confirmed in most cases. For 53 participants seen by a single counselor, 45 (85%) couples agreed to prenatal testing, and 33 of those 45 (74%) agreed to add a-CGH as a testing method. Reasons for accepting a-CGH testing included a previous affected child or an abnormal ultrasound, while rarity of the disorders tested and concern for an increased level of anxiety that may be raised by the test were reasons for declining. This study demonstrated a high level of acceptance in this population already undergoing amniocentesis or CVS, a high level of accuracy when compared to the standard karyotype, and a relative low level of results requiring additional testing to clarify clinical significance.

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An algorithm for cgh microarray in clinical practice. *E. Aston, H. Whitby, T. Maxwell, B. Milash, B. Issa, J. Xu, Z. Chen, A. Brothman.* University of Utah Health Sciences Center, Salt Lake City, UT.

High resolution genomic (cgh) microarrays to detect copy number changes have been used in research since 1998. Several commercial platforms are available for use of this technology, and it has recently been applied to diagnostics. The University of Utah Cytogenetics laboratory has used both the SpectralChip 2600 (1Mb) and the Constitutional Chip from Spectral Genomics Inc. (Houston, TX) since 2003 for research and validation, and began clinical testing in January 2005. While the techniques are relatively new, results have shown promise in detection and detailed characterization of cytogenetic aberrations. An algorithm has been defined to determine which chip is used and how testing is performed. With few exceptions, most clinical studies have been processed according to this formula. High resolution G-band karyotypes are required before an array is run. If an abnormality requires further characterization, the 1Mb chip is recommended, as this chip contains BAC clones which cover the human genome at ~1Mb intervals. When normal karyotypes are observed but phenotype is indicative of described genetic syndromes, the Constitutional Chip is recommended. In some circumstances, both chips may be used. Pooled normal male or female genomic DNAs are used for controls. Any detected imbalances are confirmed with FISH using the same or representative clones, and parental DNAs are analyzed when appropriate and available. To improve sensitivity, we developed a statistical model that reduces noise on the array which may be an artifact of the manufacturing process, and provides improved discrimination between normal and abnormal chromosomal regions. Recognition of copy number polymorphisms (several unreported sites identified in this lab) and interpretation of their significance remains the largest challenge in our analyses. We have used this algorithm to evaluate 40 patients to date, (17 1Mb and 23 Constitutional) and have elucidated new information in all cases. Eleven (65%) and 7 (30%) abnormalities were detected with each chip, respectively. As this technology approaches the accepted standard of care, the field must cautiously move forward in implementation.

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Case-control linkage disequilibrium difference (CCOLDD): a linkage disequilibrium mapping method in genome-wide association studies. *M.G. Hayes[1,2], N.J. Cox[1,2].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Sctn Genetic Medicine, Dept Medicine, Univ Chicago, Chicago, IL.

Multipoint linkage disequilibrium (LD) mapping methods currently available are too computationally intensive to feasibly apply to large scale genome wide association data. We have developed a much less computationally intensive visualization method, called case-control linkage disequilibrium difference (CCOLDD) for rapidly determining regions that contain differences in the extent of LD between cases and controls. The increased frequency of common, historically-related derived susceptibility allele(s) in a region reduces the haplotype diversity in cases relative to controls and increases the extent of LD. We utilize LDU maps of cases and controls over the region or chromosome of interest and calculate the slope of the LDU difference between cases and controls in windows of varying size to pinpoint regions with rapidly changing differences in the extent of LD in cases and controls. CCOLDD efficiently detected the regions harboring susceptibility alleles in two densely typed positional cloning data sets: the *CAPN10* gene in the *NIDDM1* region associated with type 2 diabetes, and the *CFTR* gene associated with cystic fibrosis. Using Affymetrix 10K SNP chip data from a genome-wide linkage and association study of persistent stutterers, regions highlighted by the CCOLDD method correspond to regions showing evidence for linkage as measured by Kong and Cox LOD scores. Regions showing sex-specific linkage also showed sex-specific differences in the case-control LD comparison. Additionally, we report here on our recent results (1) determining the impact of window size, marker density, and Hardy Weinberg disequilibrium on CCOLDD results, (2) contrasting CCOLDD results with results obtained using more computationally intensive LD mapping methods such as decay of haplotype sharing (DHS) and TreeLD, (3) investigating the consequences of ancestral vs. derived susceptibility alleles on the extent of LD in cases and controls, and (4) piloting the approach in even denser data such as the Affymetrix 100K SNP chip set.

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Combined analysis of 4500 Single Nucleotide Polymorphisms from chromosome 1q21-25 in samples from eight linked populations reveals shared type 2 diabetes susceptibility variants. *M.I. McCarthy[1], E. Zeggini[1], W. Rayner[1], C.J. Groves[1], B.D. Mitchell[2], J. O'Connell[2], S.C. Elbein[3], R. Hanson[4], L.J. Baier[4], W.C. Knowler[4], P. Froguel[5], W.P. Jia[6], M.C. Ng[7], J.C. Chan[7], L.R. Cardon[1], K. Xiang[6], C. Bogardus[4], S.E. Hunt[8], P. Deloukas[8], A.R. Shuldiner[2], International Type 2 diabetes 1q consortium.* 1) University of Oxford, Oxford, United Kingdom; 2) University of Maryland, Baltimore MD; 3) University of Arkansas for Medical Sciences, Little Rock, AR; 4) Phoenix Epidemiology and Clinical Research Branch, NIDDK, Phoenix, AZ; 5) CNRS UMR 8090 Lille, France; 6) Shanghai Diabetes Institute, China; 7) Chinese University of Hong Kong, Shatin, Hong Kong; 8) Wellcome Trust Sanger Institute, Hinxton, UK.

Chromosome 1q ranks as amongst the best replicated linkage regions for type 2 diabetes (T2D). The international T2D 1q consortium has initiated dense LD mapping of the ~20Mb region of maximal linkage in samples from eight linked populations (Amish, Utah, UK, French, Hong Kong, Shanghai, Pima Indians, African Americans). To date, ~4500 SNPs have been genotyped in 4500 individuals. A Mantel-Haenszel approach has been used to combine association results across populations. Several regions showed multiple, clustered, replicated associations. The strongest evidence for association observed to date across all populations lies around 152.1Mb, near *PKLR*, where the most-associated SNPs generate a combined odds ratio of 1.49 (95% CIs: 1.27-2.14, $p<10^{-6}$). This association is restricted to the Europid populations, is strongly supported by haplotype analyses (e.g. in European subjects, $p<2x10^{-5}$ using GENEBPM, based on 50,000 replicates), maps to a region implicated as a diabetes locus in both the GK and Torii rat and includes several strong positional candidates in addition to *PKLR* (*SCAMP3*, *CLK2*, *FDPS*). In the Chinese samples, the strongest association signals lie at ~156, 158 and 161Mb; in Pimas, the clearest signals lie around 160Mb and 163Mb. This work has highlighted several regions containing shared T2D susceptibility loci. Detailed investigation of positional and functional candidates is currently underway.

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Combining multiple susceptibility polymorphisms can increase the predictive power of genetic information: a study of replicated type 2 diabetes variants. *A.T. Hattersley[1], M.N. Weedon[1], M.I. McCarthy[2], M. Walker[3], G. Hitman[4], B. Shields[1], K.R. Owen[1], T.M. Frayling[1].* 1) Diabetes and Vascular Medicine, Peninsula Medical School, Exeter, UK; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Old Road, Headington, UK; 3) Department of Medicine, School of Medicine, Newcastle upon-Tyne, UK; 4) Department of Diabetes & Metabolic Medicine, Barts and the London, Queen Mary School of Medicine and Dentistry, University of London, UK.

Alleles of polymorphisms at the *Kir6.2* (K23), *PPARG* (Pro12), *Calpain10* (SNP44 C), *HNF4A* (P2 haplotype) and *HNF1A* (V98) genes predispose to type 2 diabetes mellitus (T2D). Individually each polymorphism only moderately increases risk (~20%) and they are thought to be unhelpful in assessing subjects risk clinically. The impact of looking at multiple alleles simultaneously is not known.

To assess the combined effect of multiple susceptibility alleles we genotyped these variants in a large case/control study (1608 controls, 2396 cases). Individual allele odds ratios (OR) ranged from 1.09 [0.97, 1.23] to 1.33 [1.15, 1.53]. We found no evidence of gene-gene interaction. Each of the SNPs was consistent with a simple multiplicative model. The allelic odds ratios at each of the SNPs did not differ significantly from each other (P = 0.22). This suggested a simple model where subjects were classified according to number of risk alleles. There was a log-linear increase in odds of diabetes with increasing number of risk alleles (Chi-Sq test for log-linear trend, P 0.0001); each additional risk allele increasing the odds of a subject having T2D by 1.16 [1.09, 1.23] times. The 2.5% of subjects with 6 risk alleles had an odds ratio of 2.21 [1.27, 3.83] against the 4% of subjects with 1 risk alleles.

In conclusion, although individual susceptibility alleles only moderately increase the risk of T2D the effects are multiplicative. Combining alleles allows the identification of subgroups of the population with markedly differing risks of developing T2D. This approach may have a role in targeting preventative approaches in the future.

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The effects of genetic variation in *PSARL* on diabetes risk. *J. Curran[1], J. Jowett[2,3], K. Elliott[2], L. Kerr-Bayles[4], S. Wanyoni[4], J. Skelton[5], K.H. Kim[5], T. Dyer[1], K. Walder[3,4], G. Collier[3,4], P. Zimmet[2,3], J. Blangero[1,3], A. Kissebah[5].* 1) Southwest Foundation for Biomedical Research, TX, USA; 2) International Diabetes Institute, Vic, AUS; 3) ChemGenex Pharmaceuticals LTD, Vic, AUS; 4) Metabolic Research Unit, Deakin University, Vic, AUS; 5) Medical College of Wisconsin, WI, USA.

Following linkage studies, the next step of identifying a disease gene from many genes in a region presents a formidable task. Our approach combines human linkage and animal model data to aid in identifying genes involved in diabetes risk. Results of differential gene expression in skeletal muscle of *Psammomys obesus* identified *PSARL*, presenilins-associated rhomboid-like protein, as a strong positional candidate in the 3q27 metabolic syndrome linkage region. Studies have supported *PSARL* as a key regulator of mitochondrial function and data strongly suggests this gene is involved in insulin resistance. To further assess the role of *PSARL* on diabetes risk, we re-sequenced 6.5kb, identifying 14 SNPs and genotyped these in 1031 Caucasian individuals from 162 families (where the original linkage signal was obtained). Fasting plasma insulin levels were used as a measure of diabetes status. Bayesian quantitative trait nucleotide analysis revealed four SNPs, A-309G, C384T, C42440T and C44233G, showing significant association with plasma insulin (p=0.0027, p=0.0024, p=0.005 and p=0.0026 respectively). In addition to influencing plasma insulin levels, C44233G showed strong evidence for a genotype-by-age interaction. A combined test of the influence of this SNP on plasma insulin levels strongly supported the hypothesis that this coding variant is either directly involved or correlated with another causal variant (p=0.00025). A promoter variant, A-309G, was identified as being in linkage disequilibrium with this coding variant. Functional analysis of A-309G in L6 myotubes has shown the promoter with the A allele has a significantly higher activity compared to the promoter with the G allele (p=0.0001). These results highlight *PSARL* as a positional candidate gene for diabetes risk and indicate a likely contribution to the 3q27 linkage signal.

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Population genetics of the metabolic syndrome. *A. Di Rienzo[1], V.J. Clark[1], Y. Qian[1], R.R. Hudson[2].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept. Ecology & Evolution, Univ Chicago, Chicago, IL.

Based on the thrifty genotype hypothesis, variants that increase susceptibility to metabolic syndrome phenotypes (i.e. type 2 diabetes (T2D), obesity, cardiovascular disease, and dyslipidemia) under modern life style conditions provided a survival advantage in past environments by increasing the efficiency of energy use and storage. While this framework provides a good explanation for the pathophysiology and epidemiology of the metabolic syndrome, no population genetic model has been proposed in connection with this evolutionary hypothesis. Our previous work on candidate genes for T2D and hypertension suggested that, at least in some cases, ancestral alleles are the ones that increase risk to disease. This suggests a testable population genetic model in which thrifty alleles were ancestral adaptations maintained in ancient human populations by purifying selection. We investigated this model by population genetics simulations to compare the expected patterns of LD around ancestral vs. derived alleles for a range of frequencies. We also characterize the expected association signal for ancestral risk alleles. Additionally, we characterize empirical patterns of variation in candidate genes for the metabolic syndrome, e.g. *KCNJ11*, *USF1*, *HNF4A*, *GAD2*, and *APM1*. A trend emerges from the analysis of these genes. For variants in which the ancestral allele increases disease risk, the derived protective alleles tend to be advantageous as they appear to carry the signature of natural selection. However, for those variants in which the derived allele increases disease risk, these alleles behave as neutral. Interestingly, in some genes, different derived alleles appear to carry the signature of positive selection in different populations. This raises the possibility that natural selection acting on derived protective alleles may generate relatively high levels of inter-ethnic diversity of disease variants. Overall, our simulations and empirical studies indicate that a broader evolutionary framework may aide in the design and analysis of SNP-based association studies as well as suggest approaches to identify novel, interesting variants for future study.

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GENOMEWIDE HAPLOTYPE SIGNATURES OF HYPERTENSION WITH AND WITHOUT METABOLIC SYNDROME. *P. Hamet[1], O. Seda[1], E. Merlo[2], J. Tremblay[1], U. Broeckel[4], D. Gaudet[3], G. Bouchard[7], F. Gagnon[5], G. Antoniol[6], P.L. Brunelle[1], A. Gurau[1], F. Gossard[1], J. Pintos[1], T.A. Kotchen[4], Z. Pausova[1], S.N. Orlov[1], R. Wan[1], M. Labuda[1], M. Jomphe[7], A.W. Cowley[4].* 1) CR CHUM, Montreal, PQ, Canada; 2) Ecole Polytechnique de Montreal, Montreal, PQ, Canada; 3) Complexe hospitalier de la Sagamie, Chicoutimi, PQ, Canada; 4) Medical College of Wisconsin, Milwaukee, WI; 5) University of Ottawa, , Ottawa, ON, Canada; 6) University of Sannio, Benevento, Italy; 7) UQAC, Chicoutimi, Quebec, Canada.

Hypertension (HT) is perceived as a constitutive part of the metabolic syndrome (MS), though not being its requisite in any of MS definitions. We tested a hypothesis that genomic determinants may be distinct in hypertension with and without MS. In 120 French-Canadian families from the Saguenay-Lac-St-Jean region of Quebec, Canada, we identified hypertensive families showing extremes in presentation of MS. After comparing the families using battery of >50 anthropometric, metabolic and humoral phenotypes, we genotyped 208 individuals from hypertensive families with high density of MS (HDMS, n=126) and low-density of MS (LDMS, n=84) using Affymetrix GeneChip Human Mapping 50K Array to identify the HT genomic signatures (specific haplotype sets) in the respective family sets. Using a method of layered founders, we assessed the separability of HDMS and LDMS families in terms of specific ancestry. The LDMS families showed clearly distinct clinical features in comparison to HDMS, e.g. significantly lower volume of extracellular water. In LDMS families, the HT genomic signatures were associated with genomic determinants of blood pressure on chromosomes (chr.) 2, 5, 6 and 10. In contrast, in HDMS families HT is highly predicted based on haplotypes related with mostly metabolic MS attributes on chr. 1, 3, 7, 14, 15 and 18. At the depth of 14 layers/generations, the ancestors of LDMS families showed a higher specificity of genetic contribution to the recent generation in contrast with HDMS (84% vs. 60%). Using a genomewide SNP typing approach combined with genealogical analyses, we have identified specific genomic signatures of distinct forms of hypertension - with and without features of MS.

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The RET Gene is a modifier of the PHOX2B gene for the Hirschprung disease phenotype. *L. de Pontual, A. Pelet, D. Trochet, Y. Espinosa-Parrilla, A. Munnich, F. Jaubert, J. Feingold, S. Lyonnet, J. Amiel.* Genetics, Hopital Necker, Paris, France.

Hirschsprung disease (HSCR, MIM 164761) is a frequent congenital malformation of the hindgut defined by the absence of enteric neurons. HSCR stands as a model in the study of diseases with a complex mode of inheritance. When observed as an isolated malformation, the transmission is non mendelian with low sex-dependant penetrance. A multiplicative oligogenic model with 3 loci has been proposed with RET proto-oncogene being the key player. Indeed, almost all HSCR patients harbor either a heterozygous mutation of the coding sequence or, more frequently, a hypomorphic allele located in a conserved non gene sequence in intron 1. In roughly 30/100 of the cases however, HSCR is associated to other malformations. Hitherto, the disease causing gene is known in 4 syndromic HSCR forms with mendelien inheritance among which CCHS (MIM209880, PHOX2B mutation) and Mowat-Wilson (MIM235730, ZFHX1B mutation). The penetrance of the HSCR phenotype is estimated about 20/100 for PHOX2B gene mutation and 60/100 for ZFHX1B mutation. To test whether RET could be regarded as a modifier gene for the enteric phenotype in these 2 syndromes, we genotyped the RET locus in patients for which the disease-causing mutation had been identified previously for CCHS (N=143) or MWS (N=30). Splitting patients into 2 groups (with or without HSCR) for each syndrome showed a statistically significant over representation of the RET hypomorphic allele in CCHS patients only (=11.52; $p<0.001$). Therefore RET acts as a modifier gene for the enteric phenotype to occur in patients with a PHOX2B gene mutation. The study of a family where a PHOX2B mutation segregates with neuroblastome predisposition in one branch and HSCR in another branch further strengthen the role of the RET gene for the enteric phenotype to occur. These data illustrate the concept of a developmental gene being either a major disease-causing gene or a modifier gene depending on the clinical context. Finally, we also suggest the possibility of RET dependent and RET independent HSCR cases.

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Differential liabilities of coding and non-coding mutations in complex disease. *G. Burzynski[1], J. Amiel[2], G. Antinolo[3], S. Borrego[3], I. Ceccherini[4], E. Emison[5], C. Eng[6], R. Fernandez[3], M. Garcia-Barcelo[7], P. Griseri[4], R. Hofstra[1], C. Kashuk[5], F. Lantieri[4], S. Lyonnet[2], P. Tam[7], A. Tullio-Pelet[2], K. West[5], A. Chakravarti[5].* 1) Dept Medical Genetics, Univ Groningen, The Netherlands; 2) UC Genetica y Reproduccion, HH UU Virgen del Rocio, Seville, Spain; 3) Dept Genetique et Unite INSERM U-393, Paris, France; 4) Lab di Genetica Molecolare, Ist Gaslini, Genova, Italy; 5) Inst Genetic Medicine, Johns Hopkins Univ Baltimore, USA; 6) Dept Molecular Genetics, Ohio State Univ, Columbus, USA; 7) Dept Surgery, Genome Research Centre, Univ Hong Kong, China.

Hirschsprung disease (HSCR), a developmental disorder characterized by absence of enteric ganglia in the gut, is multifactorial. RET plays a central role; high-penetrance RET coding sequence (CDS) mutations occur in ~50%; of familial HSCR and linkage has been found in ~90%; of families. To determine whether RET non-coding sequences (NCDS) mutations contribute to risk, we studied 876 HSCR families from three continents. A common, disease-associated haplotype was constructed at the 5 RET region in all populations. The largest contribution to risk was made by an enhancer mutation in intron 1. HSCR risk varies by segment length affected (S/L, short/long; TCA, Total Colonic Aganglionosis), gender and the finding of CDS mutations. The NCDS mutation makes a greater contribution to susceptibility for S-HSCR than for the most severe TCA. Conversely, CDS mutations are detected more frequently in TCA than in S-HSCR. We also tested the contributions of CDS and NCDS mutations by proband gender as the incidence of HSCR is 4:1 (M:F). The NCDS variant makes a bigger contribution to risk in boys than in girls, while female HSCR patients carry more CDS mutations. We suggest a model in which the enhancer mutation contributes more to disease liability in the most frequent presentations of disease (male, S-HSCR, CDS mutation negative) and the rare, CDS mutations make the greatest contributions to the less frequent manifestations of HSCR (female, TCA). The types and parental origin of mutation is also skewed. Our data provide a consistent explanation of the genetic features of a classical multifactorial disease.

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A dense genome SNP scan identifies a common determinant of obesity. *A. Herbert[1], N.P. Gerry[1], M. McQueen[2], I. Heid[3,4], A. Pfeufer[5,6], T. Illig[3,4], H.-E. Wichmann[3,4], T. Meitinger[5,6], X. Zhu[7], R. Cooper[7], K. Ardlie[8], H.N. Lyons[9], J.N. Hirschhorn[9], N.M. Laird[2], M.E. Lenburg[1], C. Lange[2], M.F. Christman[1].* 1) Dept Genetics & Genomics, Boston University School of Medicine; 2) Dept of Biostatistics, Harvard School of Public Health; 3) KORA Group, GSF National Research Center, Neuherberg; 4) Institute of Epidemiology, GSF National Research Center, Neuherberg; 5) Institute of Human Genetics, Technical University Munich, Munich; 6) Institute of Human Genetics, GSF National Research Center, Neuherberg; 7) Loyola University Medical Center; 8) Genomic Collaborative Inc; 9) Program in Medical and Population Genetics, Broad Institute of MIT and Harvard.

Obesity contributes to the risk of common diseases such as diabetes, heart disease, metabolic syndrome and hypertension and is estimated to contribute to over 100,000 premature deaths in the United States annually. Heritability of BMI has been estimated to be in the range of about 50%. Despite this, common genetic determinants of obesity risk are largely unknown. To identify human genes affecting body mass index, a surrogate measure of obesity, we genotyped and analyzed 86,604 sites of common genetic variation using Affymetrix 100K Genechips in 694 participants from families in the NHLBI-Framingham Heart Study Offspring Cohort using the public release dataset. Employing a novel family-based screening procedure (Van Steen et al., 2005), a SNP (MAF =0.35)was identified that had a genome-wide significant association with elevated BMI. The association has been replicated in the MONICA/KORA population-based study of 4000 unrelated individuals of Western European ancestry (p=0.013), in a case-control study of 3000 individuals of European ancestry from the US and Poland (p = 0.01), and in a combined family-based and case-control study of 1000 African-Americans. The predisposing homozygous genotype is present in about 10% of individuals and may explain up to 4% of the population-attributable risk of obesity. The findings support the common disease/common variant hypothesis for BMI and illustrate the power of genome-wide family-based association studies to uncover genetic determinants of common phenotypes and disease.

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A Network Approach to Genetic Studies of Complex diseases. *X. Zhou[1], F. Arnett[1], M. Xiong[2].* 1) Dept Internal Medicine, Univ Texas, Houston, Houston, TX; 2) Human Genetics Center, University of Texas - Houston, Houston, TX.

Revealing mechanisms underlying complex diseases poses great challenges to biologists. The traditional linkage and linkage disequilibrium analysis that have been successful in the identification of genes responsible for Mendelian traits, however, have not led to similar success in discovering genes influencing the development of complex diseases. Emerging functional genomic and proteomic (Omic) resources and technologies provide great opportunities to develop new methods for systematic identification of genes underlying complex diseases. In this report, we propose a systems biology approach, which integrates omic data and identify hierarchically organized network structure of biochemical pathways, to find genes responsible for complex diseases. This approach consists of five steps: (1) generate a set of candidate genes using gene-gene (or protein-protein) interaction data sets; (2) reconstruct a genetic network with the set of candidate genes from gene expression data and calculate gene flows in the network; (3) identify differentially regulated genes between normal and abnormal samples in the network based on distributions of gene flows in the genetic network; (4) validate regulatory relationship between the genes in the network by perturbing the network using RNAi and monitoring the response using RT-PCR; and (5) genotype the differentially regulated genes and test their association with the diseases by direct association studies. To prove the concept in principle, the proposed approach is applied to genetic studies of the autoimmune disease scleroderma or system sclerosis which resulted in identifying SPARC gene in TGF beta pathway.

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Discovering human diseases genes with the help of mouse models. An example with Dup(17)(p11.2p11.2) syndrome. *K. Walz, J. Yan, W. Bi, R. Paylor, J.R. Lupski.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Genomic disorders are a result of recurrent DNA rearrangements involving unstable genomic regions, and are frequent diseases (~1 per 1,000 births). In a subset of such conditions the rearrangements comprise multiple unrelated contiguous genes that are physically linked(contiguous gene syndromes, CGS). Smith Magenis syndrome (SMS) is a CGS associated with a microdeletion within chromosome 17 band p11.2. The birth prevalence is estimated at 1/25,000. The clinical phenotype includes craniofacial abnormalities, brachydactyly, self injurious behavior, seizures, sleep abnormalities and mental retardation. Molecular studies revealed a common deleted region of ~4 Mb in 70-80% of SMS patients. A duplication of the same genomic region gives an independent CGS called Dup(17)(p11.2p11.2). Patients with Dup(17)(p11.2p11.2) are underweight, with hyperactivity and learning disabilities. Importantly, due to the small number of patients aviable, and that the size of the duplication seems to be the same in all of them, the discovery of the gene(s) responsible for this syndrome utilizing human patients is extremely improbable. Recently, mutations in the retinoic acid induced 1 gene (RAI1) have been identified as causative of SMS, however, the gene responsible for Dup(17)(p11.2p11.2) syndrome remains elusive. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. By chromosome engineering we have generated a mouse model for Dup(17)(p11.2p11.2), Dp(11)17/+. Dp(11)17/+ mice are underweight and have hyperactivity and learning disabilities recapitulating the human phenotype. As Rai1 is a dosage sensitive gene present in this genomic interval we wanted to test the hypothesis that Rai1 is the gene responsible for the phenotypes observed in Dp(11)17/+. By mating Dp(11)17/+ with mice carrying a mutation in the Rai1 gene: Rai1+/-, we obtained compound heterozygote Dp(11)17/Rai- animals, in which the normal gene dosage of Rai1 is obtained despite the segmental duplication. In these mice a normal weight and learning behavior was observed, suggesting that Rai1 is the predominant responsible gene for the phenotypes observed in Dp(11)17/+ mice.

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Strategies for detection of chromosome abnormalities in children with developmental delay: is it effective to use subtelomere FISH analysis as an initial screening test? *J.B. Ravnan[1], J.F. Stefanik[1,2], G. Avery[1], J.T. Mascarello[1].* 1) Genzyme Genetics, Santa Fe, NM; 2) University of Connecticut.

Clinical geneticists are faced with an array of choices for genetic testing of children with developmental delay or mental retardation. Without specific features to suggest a particular single gene disorder, most geneticists will pursue chromosome analysis followed by subtelomere FISH testing if the chromosome results are normal. Because subtelomere FISH testing can show abnormal results for large chromosome rearrangements as well as for cryptic rearrangements of the ends of the chromosomes, the question has been raised whether it might be effective to start with subtelomere FISH studies and then pursue chromosome analysis if needed. In order to find out what proportion of chromosome abnormalities detected by traditional G-banding could be detected by FISH with a subtelomere probe panel, five years of blood chromosome data from the Genzyme Genetics Santa Fe laboratory were reviewed. Abnormal chromosome results were reviewed and classified as detectable or undetectable by a subtelomere FISH panel. Preliminary review shows that approximately 80% of abnormal chromosome results overall would be detectable by subtelomere FISH, but only 50% of abnormal chromosome results found in children referred for developmental delay or mental retardation would be detectable by subtelomere FISH. Further discussion of these results will be presented and the efficacy of different testing strategies will be outlined.

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Systematic screening for microduplications / microdeletions in patients with mental retardation using a simple QMPSF assay. *P. Saugier-Veber[1,2], A. Goldenberg[1], V. Drouin-Garraud[1], V. Layet[3], N. Drouot[1,2], G. Joly-Helas[4], C. de la Rochebrochard[4], H. Moirot[4], M. Tosi[2], T. Frebourg[1,2].* 1) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Inserm U614, Faculty of Medicine, 22 boulevard Gambetta, 76183 Rouen, France; 3) Department of Cytogenetics, Le Havre Hospital, 76083 Rouen, France; 4) Department of Cytogenetics, Rouen University Hospital, 76031 Rouen, France.

Microduplications, like microdeletions, result from illegitimate recombinations mediated by repeat elements, but their frequency and clinical presentation remain to be characterized. In order to evaluate the frequency of such microduplications, we developed a new one tube QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) assay covering loci either involved in microdeletion syndromes or known to be prone to genomic rearrangements. This microdeletion / duplication QMPSF assay includes 12 amplicons, corresponding to *WHSC1* (4p16.3), *SEMA5A* (5p15.2), *NSD1* (5q35), *ELN* (7q11), *EXT1* (8q24.13), *WT1* (11p13), *SNRPN* (15q11), *CREBBP* (16p13.3), *RAI1* (17p11.2), *LOXL1* (15q22), *PAFAH1B1* (17p13.3) and *TBX1* (22q11) and was used to screen 2 series of patients. In the first series, we screened 106 patients with mental retardation (MR) and facial dysmorphism associated with malformations, or growth anomalies, or familial history. Additional criteria were: cytogenetically normal chromosomes, no detectable FRAXA mutation, and no detectable subtelomeric rearrangement. A deletion affecting *NSD1* at 5q35 was identified in a patient with severe epilepsia, profound MR and, retrospectively, craniofacial features suggestive of Sotos syndrome. In the second series, we screened 101 patients with MR and behaviour disturbance who did not fulfil the de Vries criteria for subtelomeric deletion screening. We identified a 22q11 deletion in a patient with moderate MR, obesity and facial dysmorphism, and a 17p11 duplication in a patient with mild to moderate MR, behaviour disturbance, strabism and non specific facial features. Our results suggest that patients with MR should be screened for these rearrangements regardless of their inclusion or not in subtelomeric deletion screening.

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Duplication of the commonly deleted Williams-Beuren syndrome region at 7q11.23 causes severe expressive language delay. *L.R. Osborne[1], C.B. Mervis[2], E.J. Young[1], E.J. Seo[3], M. del Campo[4], S. Bamforth[5], E. Peregrine[2], W. Loo[1], M. Lilley[5], C.A. Morris[6], L.A. Pérez-Jurado[4], S.W. Scherer[3], M.J. Somerville[5].* 1) Medicine, University of Toronto, Toronto, ON, Canada; 2) Psychological and Brain Sciences, University of Louisville, Louisville, KY; 3) Genetics & Genomic Biology, Sick Kids Hospital, Toronto, ON, Canada; 4) Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain; 5) Medical Genetics, University of Alberta, Edmonton, AB, Canada; 6) Pediatrics, University of Nevada School of Medicine, Las Vegas, NA.

The Williams-Beuren syndrome (WBS) locus at 7q11.23 is prone to recurrent rearrangements mediated by complex low copy repeats (LCRs) that flank the region. Deletions span a common 1.5 million base pair interval and result in WBS, a multi-system developmental disorder with characteristic cardiovascular, cognitive and behavioural features. It had been hypothesized that duplications of the WBS region should arise at equal frequency, although to date none have been identified. Here we present the first such case of reciprocal duplication of the WBS region. The most striking phenotype in the proband was severe developmental apraxia of speech, which is in sharp contrast to individuals with WBS who have normal articulation and fluent expressive language. The duplication breakpoints mapped within the same blocks of the LCR as those of common WBS deletion, indicating that this duplication is indeed the reciprocal of the deletion. Genes within the minimal critical interval for WBS showed increased expression in the proband, and reduced expression in individuals with WBS, compared to individuals with non-rearranged chromosomes. This is the first example of a novel syndrome due to duplication of the WBS region. WBS is characterized by relatively spared expressive language and severely impaired visuospatial abilities. In striking contrast, our patient shows the converse, suggesting that specific genes at 7q11.23 are exquisitely sensitive to dosage alterations, which can influence human language and visuospatial capabilities.

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SIZN1 (Smad Interacting Zinc Finger Protein): a new gene implicated in X-linked mental retardation. *C.E. Schwartz[1], G. Cho[2], S.S. Bhat[1], Y. Lim[3], D. Zand[4], J. Gao[1], J. Collins[1], A.K. Srivastava[1], J.A. Golden[2,5].* 1) Greenwood Genetic Center, Greenwood, SC; 2) Dept Pathology, CHOP, Philadelphia, PA; 3) Dept Biology, Univ of Penn School of Med; Philadelphia, PA; 4) Dept Genetics, CHOP, Philadelphia, PA; 5) Dept Pathology, Univ of Penn School of Med, Philadelphia, PA.

SIZN1, a transcriptional co-activator located at Xq22.2, was recently identified by us. The gene derives its name as follows: Smad Interacting Zinc finger protein expressed in the Nervous system and encodes a 402 amino acids protein containing a CCHC domain and a putative nuclear localization sequence. *SIZN1* positively regulates BMP signaling in the nucleus through interaction with Smads that recruit CREB-Binding Protein (CBP). The expression pattern and location on the X chromosome indicated that *SIZN1* might be a candidate gene for X-linked mental retardation (XLMR). The coding region of *SIZN1* was screened in a cohort of 540 males with non-fragile X MR using IPS. All abnormal alleles were directly sequenced. Two distinct mutations were identified. Four brothers with MR had a c.1031C>T (p.T344I) mutation and the mother was heterozygous for this missense mutation. This allele was not observed in 430 control males. A second mutation, c.19C>T (p.R7C), was identified in 1.4% (4/290) of black males with mental retardation. The mutant allele was found in 2.9% (5/172) of black female newborn controls and 3.8% (5/131) of black female controls with normal intelligence. The mutation was not found in a screen of male controls with normal intelligence (95 African-Americans and 346 Caucasians), nor was it found in 462 male newborns (223 African-Americans and 239 Caucasians). A study of 250 Caucasian males with mental retardation and 405 Caucasian females with normal intelligence did not reveal the mutation. Thus, this mutation appears to be limited to the African-American population. However, based on the number of individuals studied it is unlikely that this represents a rare polymorphism. Additionally, reporter assay studies of the p.R7C mutation revealed a 40-60% reduction in SIZN1 function. All together these data suggest a possible role for *SIZN1* in brain development and human cognitive function.

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The CC2D1A gene representing a new gene family with C2 domains controls human cognition. *L. Basel-Vanagaite[1,2], R. Attia[3], M. Yahav[3], R.J. Ferland[4], L. Anteki[3], C.A.. Walsh[4], T. Olender[5], R. Straussberg[2,6], N. Magal[2,3], E. Taub[1], V. Drassinover[3], A. Alkelai[3], D. Berkovich[7,8], G. Rechavi[2,9], A.J. Simon[2,9], M. Shohat[1,2].* 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 3) Felsenstein Medical Research Center, Petah Tikva, Israel; 4) Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, and Department of Neurology, Harvard Medical School, Boston, MA, USA; 5) Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel; 6) Neurogenetic Clinic, SCMCI, Petah Tikva, Israel; 7) Human Molecular Genetics & Pharmacogenetics, Migal - Galilee Bio-Technology Center, Kiryat-Shmona, Israel; 8) Tel Hai Academic College, Upper Galilee, Israel; 9) Sheba Cancer Research Center, Institute of Hematology, The Chaim Sheba Medical Center, Tel Hashomer, Israel.

The molecular basis of autosomal recessive nonsyndromic mental retardation (ARNSMR) is poorly understood due to the difficulty in grouping families for linkage analysis. Only two autosomal genes, PRSS12 on 4q26 and CRBN on 3p26, have been shown to cause ARNSMR, each gene in only one family. We have identified a protein-truncating mutation in the gene CC2D1A on chromosome 19p13.12 in nine consanguineous families with severe ARNSMR. CC2D1A is a member of a previously uncharacterized gene family that carries two conserved motifs, a C2 domain and a DM14 domain. The C2 domain is found in proteins which function in calcium-dependent phospholipid binding or in membrane trafficking. The DM14 domain is unique to the CC2D1A protein family, and its role is unknown. CC2D1A protein has many orthologues in mammals, as well as in other vertebrates and invertebrates. The gene was duplicated in vertebrates. CC2D1A is a putative signal transducer participating in regulation of I-B kinase/NF-B cascade. We demonstrated expression of CC2D1A mRNA in the embryonic ventricular zone and cortical plate in staged mouse embryos, persisting into adulthood with highest expression in the cerebral cortex and hippocampus. This suggests that previously unknown signal transduction pathway is important in human cognition.

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Homozygous deletion of the very-low density lipoprotein receptor gene causes autosomal recessive cerebellar hypoplasia with cerebral gyral simplification. *K.M. Boycott[1], S. Flavelle[1], A. Bureau[1], H.C. Glass[2], T.M. Fujiwara[3], E. Wirrell[2], K. Davey[1], A.E. Chudley[4], J.N. Scott[5], D.R. McLeod[1], J.S. Parboosingh[1].* 1) Dept Med Genet, Alberta Children's Hospital, Calgary, AB, Canada; 2) Div Ped Neuro, ACH, Calgary, AB, Canada; 3) Dept Hum Genet Med, McGill University, Montreal, QC, Canada; 4) Sec Genet Metab, Children's Hospital, Winnipeg, MB, Canada; 5) Dep Rad, Foothills Hospital, Calgary, AB, Canada.

The Hutterites are an endogamous German-speaking people that originated from the Anabaptist movement during the 16th century. A syndrome of nonprogressive cerebellar ataxia with mental retardation was described in the Hutterite population in the 1980s and was referred to as Dysequilibrium syndrome (DES) (AJMG 1981;9:43; AJMG 1986;22:567). We recently reviewed the clinical features and neuroimaging of 12 Hutterite patients with Dysequilibrium syndrome (DES-H). DES-H is characterized by global developmental delay, moderate to severe mental retardation, late ambulation (after age six), truncal ataxia and a static clinical course. The neuroimaging is characterized by hypoplasia of the inferior portion of the cerebellar hemispheres and vermis and mild simplification of cortical gyri. An identity-by-descent mapping approach was used to localize the gene for DES-H. Multipoint linkage calculations provided statistical evidence of linkage to 9p24. Haplotype analysis identified familial and ancestral recombination events and refined the minimal region to a 2 Mb interval between D9S129 and D9S1871. A 199 kb homozygous deletion encompassing the entire very low density lipoprotein receptor (*VLDLR*) gene was present in all affected individuals. This deletion was characterized by a STS-walking strategy and an 8-bp direct repeat, GAAAACAA, was identified at each breakpoint. VLDLR is a receptor for Reelin and the Reelin pathway guides neuroblast migration in the cerebral cortex and cerebellum, thus providing an explanation for the cerebellar hypoplasia and mild cerebral gyral simplification characteristic of this syndrome. This condition represents the first lipoprotein receptor human malformation syndrome and the second human disease associated with a Reelin pathway defect.

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***DOCK8* - A Candidate Mental Retardation Gene Located in Subtelomeric 9p.** *B.L. Griggs[1,2], S. Ladd[1], R.A. Saul[1], B.R. DuPont[1,2], A.K. Srivastava[1,2].* 1) Greenwood Genetic Center, Greenwood, SC; 2) Department of Genetics and Biochemistry, Clemson University, Clemson, SC.

Submicroscopic aberrations in the subtelomeric regions of the autosomes have been found in 5-10% of patients with mental retardation and developmental disabilities (MR/DD). To facilitate autosomal MR gene identification, we have previously mapped a critical genomic region associated with MR in two unrelated patients and chromosomal rearrangement involving 9p subtelomere. In one patient, a male with MR, no speech, seizures, and some dysmorphic facial features, we mapped a genomic deletion of approximately 230 kb in subtelomeric 9p. In the second patient, a female with MR and ectodermal dysplasia and a balanced translocation t(X;9)(q13.1;p24), we mapped the 9p24 breakpoint to a region overlapping with the centromeric end of the 230 kb subtelomeric deletion. The ectodermal defects in the female patient are due to the disruption of the EDA gene at Xq13.1. Thus, the MR in the patient with the translocation is presumably associated with the 9p24 breakpoint.

In silico analysis of the gene content of the critical subtelomeric MR region revealed two known genes, *FOXD4* and *CBWD1*, and a putative partial gene, *DOCK8*. We assembled and characterized the novel *DOCK8* gene from the corresponding expressed sequences in the database. Several isoforms of the *DOCK8* gene have been identified. The longest isoform of the *DOCK8* gene was found to be disrupted in both patients. The gene was partially deleted at its 5 end in the patient with the subtelomeric 9p deletion and disrupted by the 9p24 translocation breakpoint in the second patient. The gene is expressed in several tissues including adult and fetal brain. All together these findings indicate that haploinsufficiency of DOCK8 is likely responsible for MR in the two patients with subtelomeric 9p aberrations and suggest a putative role for DOCK8 in brain development and function. Further analysis of the *DOCK8* gene in unrelated patients with MR, as well as functional analysis, should clarify a possible involvement of DOCK8 in the etiology of MR.

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Analysis of the STK9/CDKL5 gene in patients with suspected Rett syndrome and X-linked infantile spasms syndrome/West syndrome. *A. Alkhateeb, K.L. Clift, K.S. Petras, W.B. Dobyns, S. Das.* Department of Human Genetics, University of Chicago, Chicago, IL.

The STK9 (or CDKL5) gene that codes for a putative kinase has recently been implicated in patients with a wide range of clinical presentations that include seizures, infantile spasms, developmental delay, mental retardation, hypotonia, absent speech, ataxia and stereotypic hand movements. The phenotypes observed in patients with STK9 mutations have included cryptogenic infantile spasms (ISS or West syndrome) and atypical Rett syndrome. Recent studies have demonstrated the interaction of STK9 with MECP2, implicating them in the same molecular pathway. While seizures appear to be a common feature in patients with STK9 mutations, no clear genotype-phenotype correlations have been reported. We set out to study involvement of the STK9 gene in a large group of patients that were refered to our molecular diagnostic laboratory, or our brain malformation research project, for testing of ARX or MECP2. We selected 95 patients with ISS (83% male) negative for mutations of ARX, and 127 patients with Rett syndrome-like features (69% female) negative for mutations and deletions of the MECP2 gene. Informed consent was obtained from the parents or guardians of all subjects. While limited clinical information was available for most patients, seizures were listed as an indication for testing in the majority of patients with ISS and in approximately 20% of patients referred for testing of MECP2 for possible Rett syndrome (likely to be higher). Mutation analysis of STK9 by full gene sequencing is in progress in these two cohorts. To date 15 patients in the MECP2 negative group and 10 patients in the ARX negative group have been analyzed and no obvious pathogenic mutations have been identified. This study is still in its preliminary stages and results of our completed study will be presented along with detailed clinical information of mutation positive cases. This study will aid in a better understanding of the genotype-phenotype correlation in patients with STK9 mutations as well as help to better determine the prevalence of mutations in this gene in a diagnostic setting.

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Autosomal dominant mental retardation resulting from mutations in cell-adhesion molecules. *A.K. Srivastava[1], K. Bhalla[1], G.F. Guzauskas[1], T. Buchan[2], M.A. Beachem[1], S. Ladd[1], S.J. Bratcher[1], B.R. DuPont[1], R.J. Schroer[1], J. Balsamo[2], J. Lilien[2].* 1) Greenwood Genetic Center, Greenwood, South Carolina; 2) Department of Biological Sciences, University of Iowa, Iowa City, Iowa.

Mental retardation (MR) is the most common developmental disability, affecting cognitive function in about 1-3% of the human population. Genes that cause inherited MR are presumably distributed throughout the human genome. Several syndromic and nonsyndromic MR genes, especially on the X chromosome, have been cloned but success in identifying autosmal MR genes has been limited. Cell-adhesion molecules of the cadherin and immunoglobulin (Ig) superfamilies play critical roles in development of the brain, as well as maintaining synaptic structure, function and plasticity. We have identified defects of two genes encoding such cell-adhesion molecules, *CDH15* (cadherin superfamily) and *KIRREL3* (Ig superfamily) in autosomal dominant MR. Both genes are disrupted by a balanced translocation in a female patient with MR. In addition, among a group of 647 unrelated patients (552 females and 95 males) with unexplained MR, 5 female patients have missense mutations in *CDH15* and 5 other patients, one male and 4 females, have missense mutations in *KIRREL3*. All of the mutations in the expressed CDH15 protein are in the most distal EC1 repeat of the ectodomain and significantly reduce its ability to mediate cell-cell adhesion without affecting cell surface expression. Both genes are expressed in fetal and adult brain. These findings suggest a possible novel role for CDH15 and KIRREL3 in developing brain which is critical for the development and maintenance of human cognition.

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Mutations in GLUT10/SLC2A10, a facilitative glucose transporter, cause arterial tortuosity syndrome. *PJ. Coucke[1], A. Willaert[1], B. Callewaert[1], MW. Wessels[2], GM. Mancini[2], J. De Backer[1], JE. Fox[3], M. Kambouris[4], R. Gardella[5], S. Barlati[5], M. Colombi[5], HC. Dietz[6], B. Loeys[6], PJ. Willems[7], A. De Paepe[1].* 1) Univ. Hospital Ghent, Belgium; 2) Erasmus Medical Centre, Rotterdam, The Netherlands; 3) Schneider Children's Hospital,A. Einstein College of Medicine, NY, USA; 4) Yale Univ., New Haven, USA; 5) Div. Biology and Genetics, Univ. Brescia, Italy; 6) McKusick-Nathans Institute, J. Hopkins, Baltimore, USA; 7) GENDIA, Antwerp, Belgium.

Arterial tortuosity syndrome (ATS) is a rare autosomal recessive disorder characterized by tortuosity, elongation and aneurysm formation of the major arteries and pulmonary artery stenosis due to disruption of elastic fibres of the media. Homozygosity mapping in two consanguineous families, respectively from Morocco and Italy, identified a critical region of 4.1 Mb on chromosome 20q13.1. Haplotype sharing between 2 Moroccan families originating from the same region reduced the candidate interval to 1.2 Mb containing 7 genes (SLC13A3, TP53RK, SLC2A10, EYA2, PRKCBP1, NCOA3, SULF2) and 1 pseudogene (RPL35AP). Mutations in GLUT10/SLC2A10, a member of the facilitative glucose transporters (GLUTs), were identified in 6 ATS families: homozygous nonsense mutations or base pair deletions leading to premature stop codons in 4 families, and one identical missense mutation in 2 other families. Identification of a deficient glucose transporter as the underlying cause for arterial tortuosity is fascinating since glucose metabolism has not previously been involved in the elastic fibre network. Some evidence suggests that glucose influences TGFbeta signalling, a pathway associated with arterial tortuosity in the Loeys-Dietz syndrome, an aortic aneurysm syndrome caused by mutations in the TGFBR1 or TGFBR2 genes. Alternatively, GLUT10 could be involved in the transport of metabolites necessary for the synthesis or processing of glycoaminoglycans, important constituents of arterial walls. At present, other mechanisms explaining the role of GLUT10 cannot be excluded and research is ongoing to unravel the specific pathogenetic mechanisms underlying ATS.

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Loss of function mutations in the *Growth differentiation factor - 1* (*GDF1*) are associated with congenital heart defects in humans. *J.D. Karkera[1], S.J. Lee[2], S. Banerjee-Basu[3], E. Roessler[1], M.V. Ouspenskaia[1], E. Goldmuntz[4], P. Bowers[5], J. Towbin[6], J. Blemont[6], A. Schier[2], A. Baxevanis[3], M. Muenke[1].* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Skirball Institute of Biomolecular Medicine and Department of Cell Biology, NYU School of Medicine, NY; 3) Genome Technology Branch, NHGRI/NIH, Bethesda, MD; 4) Divison of Cardiology, The Children's Hospital of Philadelphia, PA; 5) Department of Pediatrics, Yale University School of Medicine, New Haven, CT; 6) Divison of Cardiology, Department of Pediatrics, Baylor College of Medicine, Huston, TX.

Congenital heart defects (CHDs) originating from defects in early embryonic patterning collectively account for the largest number of birth defects in humans, with an incidence of ~8 per 1000 live births. Growth Differentiation Factor 1 (GDF1), encoding a TGF binding is essential for maintaining the Left-Right (L-R) axis asymmetry. Gdf1 murine mutants display a wide spectrum of L-R axis formation defects including visceral *situs inversus*, the abnormal positioning of the aorta and pulmonary artery (Transposition of the Great Vessels), atrial and ventricular septal defects. To determine whether GDF1 is a susceptibility gene for the CHDs, we screened a cohort of 375 unrelated individuals affected with wide spectrum of CHDs. We have identified one nonsense, seven missense mutations, and several single nucleotide common polymorphisms. Utilizing BMP7 crystal structure as the template a molecular model was generated of mature human GDF1. Here we report that all the missense mutations in the mature GDF1 domain appear to be clustered in the hydrophobic region predicting structural disruptions that might lead to loss of function. The functional effects of the identified variants using a highly conserved mature murine *Gdf1* as a chimera displayed Goosecoid expression as active readout for GDF1. All five of the human GDF1 missense mutations in the mature domain tested showed loss of function. Thus, molecular modeling and GDF1 over-expression in zebrafish suggests that the missense mutations in the mature GDF1 domain cause loss of function and contribute to disturbances of heart patterning in humans.

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Transcript profiles of fat biopsies and two independent autopsy studies support the role of allelic variants of the *USF1*-gene in cardiovascular disease. *K. Komulainen[1], J. Naukkarinen[1], M. Gentile[1], P. Pajukanta[2], J. Saarela[1], M-R. Taskinen[3], P.J. Karhunen[4], M. Perola[1], L. Peltonen[1].* 1) Dept. of Molecular Medicine, KTL, Biomedicum, Helsinki, Finland; 2) Dept. of Hum. Gen., David Geffen School of Medicine at UCLA, CA, USA; 3) Dept. of Medicine, Helsinki University Central Hospital, Helsinki, Finland; 4) School of Medicine, University of Tampere and Research Unit of Laboratory Center, Tampere University Hospital.

We recently reported an association of allelic variants of *USF1* with FCHL, a common dyslipidemia predisposing to cardiovascular disease (CVD). To investigate the functional relevance of different *USF1* alleles and the downstream targets of this transcription factor, expression studies were conducted in fat biopsies of FCHL family members. Further, the role of specific *USF1* alleles was addressed by quantitative analysis of arterial plaques in two human autopsy series.

The best associating SNP in the FCHL families is located in a 60 bp across species conserved sequence of intron 7 that we here established to bind nuclear proteins and regulate transcription in vitro. In analysis of transcript profiles from 19 fat-biopsies the putative *USF1* risk allele was associated with significant changes of transcript levels of 3 *USF1* target genes, namely *APOE*, *ABCA1* and *AGT*, highly relevant to the pathogenesis of CVD.

To further establish the role of different *USF1* alleles in quantitative changes of arterial atherosclerosis, we examined two autopsy series consisting of 700 males with sudden death in the Helsinki area during years 1981-82 and 1991-92. Detailed autopsy data included quantitative classification of atherosclerotic lesions in coronary arteries and abdominal aorta. Specific *USF1* alleles were associated with the size of fibrotic intimal lesions and the amount of calcification of the arteries and atherosclerosis of the brain (P=0.04 - <0.0001) as well as with death from ischemic heart disease (OR 5.0, 95% Cl 1.8-14.3, P=0.003). These results underline the importance of allelic variants of the *USF1* gene as a CVD risk factor at the population level and give more insight into the molecular pathogenesis of atherosclerosis.

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Common Variants Influencing the QT-interval by Genome-wide Association Analysis. *D.E. Arking[1], A. Pfeufer[2,3], W. Post[1], W.H.L. Kao[1], M. Ikeda[1], C. Kashuk[1], S. Lin[1], D. Cutler[1], S. Perz[3], H.E. Wichmann[3], S. Kaab[4], P.M. Spooner[1], T. Meitinger[2,3], A. Chakravarti[1].* 1) Johns Hopkins Medical Institutes, Baltimore, MD; 2) Institute of Human Genetics, Technical University Munich, Germany; 3) GSF National Research Center, Germany; 4) Dept of Medicine I, University of Munich, Germany.

We performed a genome-wide SNP screen to identify genetic determinants of cardiac repolarization. The QT-interval is normally distributed in the general population and has moderate heritability (0.30). To minimize false positive findings, we took a 3 stage approach. In stage 1, we selected individuals from the extremes of the QT-interval (7.5th and 92.5th percentile), normalized for heart rate, age, and sex (QTc RAS), from 2,171 women enrolled in the population-based KORA S4 survey (age 25-75 yrs). Women were selected to avoid heterogeneity due to sex difference. 103 individuals from each extreme (mean QTc RAS: 385.77.7 ms vs. 444.83.6 ms) were genotyped for ~100,000 SNPs using Affymetrix Centurion arrays. While no single SNP reached genome-wide significance, several loci exhibited multiple SNPs with large differences in allele frequency between extremes of QT-interval (p0.0001). In stage 2, the 30 most significant SNPs (all p0.0003) from the whole-genome screen were genotyped in the next 200 females from each extreme. In stage 3, SNPs that were significant at p0.01 in the combined phase 1 and 2 datasets were then screened in all KORA S4 probands (n=3,966 after exclusion criteria: AF, pacer, and pregnancy). One SNP, in intron 1 of the CAPON gene, a regulator of nNOS which plays a key role in regulation of cardiac contractility, was significantly associated with QTc RAS ($p1.84x10^{-11}$), with ~5 ms difference in QTc RAS between the two homozygous genotype groups. When stratified by sex, this SNP was significant in both males ($p4.3x10^{-5}$) and females ($p4.7x10^{-8}$). The males serve as a validation cohort, as phases 1 and 2 used only female samples. Results of this study demonstrate the feasibility and the potential of genome-wide SNP association studies for identification of novel genes for complex traits.

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Genotype-phenotype correlations in 7,000 Americans: *CRP* haplotypes are significantly associated with high plasma C-reactive protein levels in African- and Mexican-Americans. *D.C. Crawford, J.D. Smith, C. Shephard, M. Wong, L. Witrak, M.J. Rieder, D.A. Nickerson.* Dept Genome Sci, Univ Washington, Seattle, WA.

High levels of plasma C-reactive protein (CRP) are associated with an increased risk for heart disease. Given its clinical importance, it is important to understand *CRP* regulation so that novel prevention strategies can be developed. Here we explore the genetic factors that influence *CRP* regulation using two approaches. First, seven tagSNPs were selected based on linkage disequilibrium (using LDSelect; $r^2$0.64) from re-sequencing data on *CRP* designed to capture all common genetic variation (5% minor allele frequency) for European and African-Americans. Second, we re-sequenced an additional 950 chromosomes and identified two novel nonsynonymous SNPs predicted by PolyPhen as possibly damaging. These approaches encompass both the common variant/common disease and the rare variant/common disease models. Nine SNPs were then genotyped in 7,296 samples from the Centers for Disease Control and Preventions National Health and Nutrition Examination Survey (NHANES) III. NHANES III is population-based survey of American health and is one of the largest cross-sectional DNA collections of Americans (European, n=2,631; African, n=2,108; and Mexican, n=1,862) linked to extensive phenotyping. In NHANES III, we identified only two individuals heterozygous for one cSNP suggesting *CRP* cSNPs are rare in the general population. Classifying CRP levels as a dichotomous trait (1.0 mg/dl; 1.0 mg/dl), we performed univariate analyses for SNPs and haplotypes stratifying by sex and race/ethnicity. Three SNPs (790, p0.00001; 1440, p0.003; 3872, p0.002) were significantly associated in African-American females with high CRP levels. SNPs 1440 and 1421 (tagged by 790) are located in the promoter and are predicted to alter transcription factor binding. Haplotype analysis identified a strong association in Mexican-American females (p0.0001) and males (p=0.02) with high CRP levels compared with a weaker association in African-American females (p=0.04), demonstrating the possibility that a SNP-by-SNP analysis does not identify all significant associations between genetic variation and CRP plasma levels.

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Contribution of Clinical Correlates and 13 C-Reactive Protein Gene Polymorphisms to Inter-Individual Variability in Serum C-Reactive Protein Level. *S. Kathiresan[1,2], C.Y. Guo[2], M.G. Larson[2,3], R.S. Vasan[2,3], P. Gona[2,3], P.A. Sutherland[2,3], J.F. Keaney[3], C. Newton-Cheh[1,2], S.L. Musone[1], A.L. Lochner[1], J.A. Drake[1], D. Levy[2], C.J. O'Donnell[1,2], J.N. Hirschhorn[1], E.J. Benjamin[2,3].* 1) Broad Institute, Cambridge, MA; 2) NHLBI's Framingham Heart Study, Framingham, MA; 3) Boston University, Boston, MA.

Background Serum level of C-reactive protein (CRP) is a heritable complex trait that predicts incident cardiovascular disease. We investigated the clinical and genetic sources of inter-individual variability in serum CRP level in the community. **Methods and Results** In a reference panel, we constructed a dense linkage disequilibrium (LD) map for common SNPs spanning the CRP locus (1 SNP every 830 bases, 26 kilobases). A single block of strong LD spanned the CRP locus and seven tag SNPs captured common genetic variation. We studied clinical correlates of serum CRP in 3301 Framingham Heart Study (FHS) participants (mean age 61 years, 53% women). Twelve clinical covariates explained 26% of variability in CRP level with body mass index alone explaining 15% ($P<0.0001$). Thirteen CRP SNPs (tag SNPs plus previously studied variants) were genotyped in 1640 unrelated FHS participants with measured CRP level. Adjusting for clinical covariates, nine of 13 SNPs were associated with serum CRP level ($P<0.05$). To account for correlation among SNPs, we conducted stepwise selection among all 13 SNPs and found that a triallelic SNP (rs3091244) or a two-SNP proxy for it (rs1205 and rs2808630) remained associated with CRP level (stepwise $P<0.0001$, <0.0001, and 0.003, respectively). The triallelic SNP (C>A>T, allele frequencies 62%, 31%, 6%), located in promoter sequence, explained 1.4% of variation in serum CRP. Eight common haplotypes differed with respect to mean CRP level (global $P <0.0001$). Haplotypes harboring the minor A and T alleles of the triallelic SNP were associated with higher CRP level (hap $P=0.0001$ and 0.004). **Conclusions** In our community-based sample, clinical covariates explained 26% of inter-individual variation in serum CRP levels; a common triallelic SNP in promoter sequence explained an additional 1.4% of the variation.

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Admixture Mapping for Genomic Regions Linked to Hypertension in African Americans and Mexican Americans. *X. Zhu[1], J. Cohen[2], R.S. Cooper[1], H.H. Hobbs[2].* 1) Preventive Med & Epidemiology, Loyola Univ Medical Ctr, Maywood, IL; 2) Donald W. Reynolds Cardiovascular Clinical Research Center, Eugene McDermott Center For Growth and Development, University of Texas Southwestern Medical Center, Dallas, TX.

Migration patterns in modern societies have created the opportunity to use population admixture as a strategy to identify susceptibility genes. To implement this strategy, we genotyped a highly informative marker panel of 2270 single nucleotide polymorphisms in a random population sample of African Americans (N = 1743) and Mexican Americans (N = 631). We then examined the evidence for over-transmission of specific loci to cases from one of the two ancestral populations involved historically in the origin of these groups. Cases and controls were defined for hypertension based on standard clinical criteria; both case-only and case-control analyses were performed among African Americans, but only the case-control comparison was considered to be robust in the smaller Mexican American sample. Among the African Americans, the most significant results for hypertension were observed on chromosomes 1, 4, 6 and 16. These same loci were also associated with hypertension among the Mexican Americans in a case-control analysis. Significant linkage evidence was identified on chromosome 1, 6 and 16 when combining the results from the two samples. These data demonstrate the potential for admixture mapping among large cosmopolitan populations, provide incremental information about the location of susceptibility loci for complex traits. We also addressed the problems of admixture mapping due to the misspecified ancestral allele frequencies and background linkage disequilibrium.

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Tobacco smoking, *estrogen receptor gene* variation, and small low-density lipoprotein particle level. *A.M. Shearman[1], S. Demissie[2], L.A. Cupples[2], I. Peter[3], C.H. Schmid[3], J.M. Ordovas[4], M.E. Mendelsohn[5], D.E. Housman[1].*
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High levels of small low-density lipoprotein (LDL) particles are a major risk factor for cardiovascular morbidity and mortality. Both estrogens and smoking, with known anti-estrogenic effects, alter the atherogenic lipid profile. We tested for a role of interaction between smoking and *estrogen receptor alpha gene (ESR1)* variation in association with plasma concentration of atherogenic small LDL particles and LDL particle size. We studied 1727 unrelated men and women from the population based Framingham Heart Study. After covariate adjustment women who smoked and had the common *ESR1 c.454-397 TT* genotype (in 30% of women *T* was present on both chromosomes at position 397 prior to the start of exon 2) had > 1.7-fold higher levels of small LDL particles than women with the alternative genotypes (P for smoking-genotype interaction = 0.001). Similar results were obtained for three other *ESR1* variants, including *c.454-351A>G*, in the same linkage disequilibrium block. A similar substantial gender-specific result was also evident with a fifth variant, in a separate linkage disequilibrium block, in exon 4 (P = 0.003). Women who smoked and had specific, common *ESR1* genotypes had a substantially higher plasma concentration of atherogenic small LDL particles. Significant results revealed a dose dependent effect of smoking, and were evident in both pre- and postmenopausal women. The reported association has the potential to explain the risks associated with estrogen use in certain women and a recent report of association between an *ESR1* haplotype composed of *c.454-397 T* and *c.454-351 A* alleles with increased myocardial infarction, independent of the standard cardiovascular risk factors.

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Association of common variation in AGTR1 with left ventricular mass in the Framingham Heart Study. *J.N. Hirschhorn*[1,2]. 1) for the Writing Group of the Cardiogenomics Program (an NHLBI Program for Genomics Applications), NHLBI's Framingham Heart Study and the Broad Institute of Harvard and MIT; 2) Genetics/Program in Genomics, Harvard Medical School/Childrens Hospital, Boston, MA.

Left ventricular (LV) mass and related structure/function measures are heritable intermediate phenotypes for cardiovascular diseases (CVD) such as hypertension and congestive heart failure. Because of human and animal data implicating the renin-angiotensin system (RAS) in cardiac hypertrophy and dilation, we performed comprehensive association tests of LV phenotypes with common variation in RAS genes, including angiotensin converting enzyme (*ACE*), angiotensin receptor types 1 (*AGTR1*) and 2 (*AGTR2*), renin (*REN*), and angiotensinogen (*AGT*).

To characterize common variation at these genes, we genotyped a dense set of SNPs in 12 CEPH pedigrees; 206 SNPs had frequencies >5%. We then selected 83 tag and missense SNPs that capture common variation in these genes, and genotyped these SNPs in 1234 unrelated men and women from the Framingham Heart Study (FHS), in whom LV mass (LVM), wall thickness, diastolic dimension, and fractional shortening were determined by M-mode echocardiography. Multivariable regression analysis was conducted for LV phenotypes, adjusted for sex, age, height, weight, smoking, BP, and hypertension therapy. General, additive, dominant and recessive genetic models were tested. SNPs with strong associations in the initial sample were genotyped in a validation sample of individuals from families in FHS.

Seven SNPs had at least one nominal p value <0.0001 or two nominal p values <0.001 in the initial sample, and were taken forward into the validation sample. A strong nominal association between SNP rs1800766 in *AGTR1* and LVM in men (p=0.00006 in the initial sample; age/sex adjusted, additive model) was convincingly validated in the second sample (p=0.0004 for this same phenotype and model). Initial associations with LVM under related genetic models, and for LVM with rs275649 (correlated with rs1800766; see www.cardiogenomics.org), were also validated. We conclude that variation in *AGTR1* influences LV mass in men, and is thus a good candidate for influencing CVD.

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Identification of a novel locus for left main coronary artery disease. *L. Wang[1], ER. Hauser[1], SH. Svati[1], D. Seo[2], SG. Gregory[1], WE. Kraus[2], M. Pericak-Vance[1], PJ. Goldschmidt-Clermont[2], JM. Vance[1].* 1) Center for Human Genetics,; 2) Divison of Cardiology, Duke University Medical Center, Durham, NC.

Patients with left main coronary artery disease (LMD) have one of the worst prognoses for survival of coronary artery disease (CAD). We have identified a novel locus in the limbic system-associated membrane protein (LSAMP) gene associated with LMD risk, independent of classic CAD risk factors (cholesterol, diabetes, etc.). We began by using a DNA pooling technique to screen single nucleotide polymorphisms (SNPs) for association in our CAD linkage peak on chromosome 3q13. Subjects included 204 controls and 469 cases from patients who underwent cardiac catheterization (CATHGEN), which is an independent dataset from our early-onset CAD linkage probands (n=420). Allelotyping in DNA pools found a 12% allele frequency difference between older-onset CATHGEN cases and controls for SNP rs1875518 which was validated by individual genotyping. Similar associations were found in additional SNPs near rs1875518 and was confirmed in the linkage probands ($p<0.05$). Given the significant findings in both late and early-onset CAD datasets, we examined the data for association stratified by CAD index, a numerical summary of angiographic data that reflects severity of CAD. This analysis revealed a strong association with LMD ($p<0.001$), odds ratio 2.63 (95% CI: 1.43-4.83) for the marker rs1676232 in a recessive model. The associated SNPs reside within a large intron (1.6 Mb) lying between two alternative first exons (1a and 1b) of LSAMP gene, previously only described in mouse. *In silico* analysis suggested a similar exon structure for the human gene. Expression of LSAMP exon 1a was studied in 37 human aortas and found to be decreased 6.5 fold ($p<0.001$) in aortas with severe compared to mild atherosclerosis. In addition, the rs1676232 risk allele was associated with reduced expression of exon 1a ($p=0.05$) in the aortas. Given the pronounced risk associated with LMD, our findings could lead to a risk assessment tool facilitating early initiation of preventive and therapeutic strategies, and thereby reducing CAD morbidity and mortality.

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Newborn screening reveals high incidence of later-onset Fabry disease: Implications for detection of later-onset, but treatable inherited disorders. *M. Spada[1], S. Pagliardini[1], M. Yasuda[2], G. Thiagarajan[2], A. Ponzone[1], R.J. Desnick[2].* 1) Pediatrics, Univ Torino, Torino, Italy; 2) Human Genetics, Mount Sinai School of Medicine, New York, NY.

Newborn screening for Fabry disease, an X-linked recessive lysosomal storage disease, was performed by assaying -galactosidase A (-Gal A) activity in blood spots obtained from 30,129 consecutive male infants born in the Piedmont region of Italy during the 20 months from July, 2003 through February, 2005. All 34 infants (0.11%) who had blood spot -Gal A activities <20% of the normal mean (7.7 nmol/h/ml blood) were retested. Of these, 10 infants had low -Gal A activities (range 0.0 to 0.2 nmol/h/ml blood or 0.3 to 1.1 nmol/h/ml plasma and DNA sequencing identified -Gal A missense mutations, including four newborns with novel mutations (M51I, E66G, A73V, and R118C) and six infants with three previously identified missense mutations (F113L, A143T and N215S) and one splicing defect ($IVS5^{+1GT}$). Surprisingly, the incidence of Fabry disease was ~1 in 3,000 males in this population, >15 times more frequent than the previously estimated 1 in ~50,000 males. Transient expression of the missense mutations revealed that all had residual -Gal A activities (~20-40% of wild type), which were enhanced by the -Gal A-specific pharmacologic chaperones, galactose and deoxygalactonojirmycin, findings consistent with the later-onset cardiac and renal variant phenotypes. The splicing defect was previously reported in a classically affected male. Thus, screening ~30,000 newborn males revealed a ratio of 1 classically affected to 9 later-onset variants with Fabry disease. These data suggest that the later-onset phenotype is under-diagnosed among adult male patients who will develop cardiac disease and/or renal insufficiency. Clearly, recognition of these patients would permit early intervention by enzyme replacement and/or enhancement therapy. However, the high incidence of the later-onset patients raises ethical issues related to when screening should be undertaken for this disease either in the neonate or in early maturity, perhaps in conjunction with screening for other treatable adult-onset disorders.

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Perfect Sensitivity and Specificity of Plasma Catechol Measurements for Neonatal Diagnosis of Menkes Disease.

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Background: Menkes disease (MD) is an X-linked recessive neurodevelopmental disorder that features abnormal incorporation of copper into cuproenzymes, including dopamine--hydroxylase (DBH). Neonatal diagnosis and prompt treatment with copper injections can prevent the mortality and morbidity associated with MD, however early detection is hindered by inadequate sensitivity and specificity of commonly available biochemical tests. Abnormal plasma catechol levels, due to DBH deficiency, has been noted in MD. The present study evaluated prospectively the sensitivity and specificity of these measurements as a diagnostic test for Menkes disease in at-risk infants. Methods: Plasma specimens from 81 at-risk male infants referred by hospitals and medical centers throughout the US (31 states and the District of Columbia) and 5 foreign countries were analyzed between May, 1997 and April, 2005. Thirty-six were from asymptomatic newborns with a positive family history of Menkes disease. Plasma catechols were measured by high performance liquid chromatography with electrochemical detection. Results: Of 81 specimens, 46 had an abnormal plasma catechol pattern consistent with decreased DBH activity, and 35 did not have this pattern. Among the subgroup of newborns, 14 showed the abnormal pattern and 22 did not. Clinical and molecular follow-up confirmed 100% sensitivity and 100% specificity of the abnormal catechol pattern among the entire cohort. Thirty percent of asymptomatic affected infants identified and treated from a very early age with copper histidine injections had very favorable clinical outcomes. Conclusions: Plasma catechol measurements are sensitive and specific for diagnosing Menkes disease particularly in newborn infants for whom very early recognition is a prerequisite for successful therapeutic intervention in this otherwise lethal disorder.

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Validation of pharmacogenetic data necessary to put personalized medicine into practice in rheumatoid arthritis.

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Aim: Methotrexate (MTX) and sulfasalazine (SSZ) are important drugs for the treatment of rheumatoid arthritis (RA). However, the outcome of the treatment with these drugs is known to vary among patients. We have previously reported an association between the C677T and A1298C polymorphisms of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and MTX toxicity or efficacy (Pharmacogenetics 2002;12:2525). We also indicated that toxicity of SSZ is associated with diplotype configuration at N-acetyltransferase 2 (NAT2) gene (J Rheumatol 2002;29:2492). However, another reports have produced inconsistent results. The aim of this study was to validate our previous studies. Methods: 384 RA patients were randomly selected. Genotypes of C677T and A1298C polymorphisms of the MTHFR gene and the diplotype configuration at NAT2 gene were determined. Results: 227 patients had been administered MTX. The association between toxicity of MTX and MTHFR genotypes was examined in 156 patients who received a cumulative dose of MTX more than 300mg. MTX-related toxicity occurred in 43 patients before a total cumulative MTX dose of 300mg. For C677T, toxicity was more frequent in patients with 677T than in patients without the 677T (p=0.003). MTX had been administered at starting doses 2 to 6 mg/week in 159 patients, and whether different genotypes were associated with the level of MTX dose at a year after MTX was started was examined. Patients with the 1298C required significantly lower doses of MTX than patients without the 1298C (P=0.008). 94 patients had been treated with SSZ. 33 patients experienced any adverse effect, of whom 18% carried slow-acetylator diplotype, while among the 61 patients who were free of adverse effects 5% were slow-acetylator diplotype carriers (P=0.04). Conclusion: This work confirms our previous results. The application of phamacogenetics may reduce toxicities and enhance the desirable effects of MTX and SSZ.

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A novel genome-wide approach to finding determinants of chemotherapeutic susceptibility. *S.J. Shukla[1], J.A. Badner[2], X. Wu[3], W. Zhang[1], C. Cheng[4], W.K. Bleibel[3], M.E. Dolan[3].* 1) Human Genetics, U Chicago, Chicago, IL; 2) Psychiatry, U Chicago; 3) Medicine, U Chicago; 4) Biostatistics, St. Judes, Memphis, TN.

Little is known about heritable factors that influence chemotherapeutic-associated toxicity. Our goal is to better understand the genetic basis of drug toxicity by utilizing a genome-wide approach with no a priori assumptions about candidate genes, in order to identify previously unknown genetic variation in germline DNA. Our approach combines information from linkage, expression, bioinformatics, and association analyses. Cell growth inhibition of lymphoblastoid cell lines (LCLs), derived from members of CEPH pedigrees, was quantified following treatment with a range of chemotherapeutic agents. 10 to 17 families were evaluated for linkage and heritability (H2) analyses for cisplatin, carboplatin, etoposide, daunorubicin, and vincristine. H2, using SOLAR, revealed significant values ranging from 0.24-0.73 ($p<0.0001$) for all 5 drugs. Moderate linkage peaks (lod >2) were found at chromosomes 1, 9, and 12. The 8 most resistant and 8 sensitive LCLs following cisplatin treatment were used to evaluate expression differences at baseline and across time. 829 probe sets were identified as different at baseline using ANOVA ($p<0.05$). Linear regression was used to correlate the IC_{50} (dose inhibiting 50% of cell growth) of cisplatin and publicly available baseline LCL expression data. JARID1B, a DNA binding protein, was common to linkage, baseline microarray, and linear regression analyses. Clusters of probesets that had similar patterns of expression over 0,2,5,8 hours were identified ($p<0.05$). Oct-1 emerged as a common transcription factor among a group of highly correlated probesets within a cluster, which may be important in their regulation. Pathway analysis using Ingenuity software revealed CDKN2A and MYC as central hubs of gene interactions, based on 89 genes common to linkage and microarray analyses. Phenotyped LCLs with HapMap data will be used for association. Our approach could lead to the discovery of genes that were previously unknown or unrecognized as important determinants of drug response.

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A predictive bar-code for cancer therapy based on kinase inhibitors. *A. Killian[1], R. Sesboue[1], G. Raux[1], J-M. Flaman[1], F. Le Pessot[2], F. Di Fiore[1,3], B. Paillot[3], P. Michel[3], T. Frebourg[1].* 1) Inserm U614, IFRMP, Faculty of Medicine, Rouen, France; 2) Department of Pathology, Rouen University Hospital, France; 3) Department of Gastroenterology, Rouen University Hospital, France.

The recent development of selective anticancer therapies, based on kinase inhibitors, represents one of the most important improvements in cancer treatment and recent studies have revealed that the clinical response depends on the presence of activating alterations. In solid tumours, these predictive alterations correspond to point mutations or deletions within the kinase domain but systematic screenings of tumours suggest that such alterations are infrequent. Gain of copies / amplification of genes encoding kinases appear more frequent and have also been shown to be associated with a clinical response. Therefore detection of gain of copies and of amplifications of genes encoding kinases may be required in the future for patient targeted therapies. Thus, we have developed a predictive bar-code for kinase inhibitors, called the kinogram, which is based on the QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments) method. In this single tube assay, 15 short genomic sequences (<250 pb), corresponding to genes encoding kinases against which inhibitors have been developed, and 2 control amplicons are simultaneously amplified under quantitative conditions using dye-labelled primers. For each patient, the QMPSF profile generated from the tumour is superimposed to that generated from the adjacent non tumoral tissue, using control amplicons. This predictive QMPSF bar-code was used to analyse 52 primary colorectal cancers. This preliminary study revealed that more than half of the tumours exhibited gain of copies of target genes. Furthermore, gain of copies of several targets were often detected within the same tumour. This is particularly important in the context of acquired resistance to kinase inhibitors. Indeed, as highlighted by clinical trials, the genetic instability of tumour cells represents a pitfall for targeted anti-cancer treatment. Our results suggest that in cancer, like in bacterial or viral diseases, combined targeted treatment should be of interest.

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Sequential morphology (phenotype)/ FISH (genotype) assessment by targeting plasma cells greatly improves residual disease detection in multiple myeloma. *M.L. Slovak, V. Bedell, K. Pagel, L. Weiss, G. Somlo.* City of Hope Natl Medical Ctr, Duarte, CA.

Clonal abnormities of -13/del(13q), 14q32/IGH, del(17p), and hyperdiploidy (+5, +9, +15) have been reported in >80% of newly diagnosed multiple myeloma (MM) cases. Detection of these same abnormalities post treatment by conventional cytogenetics (CC) or standard FISH, however, has proven to be very difficult in samples with less than 20% bone marrow (BM) involvement, correlating poorly with pathology assessment. In this study, we investigated 102 post treatment samples collected from 82 MM patients (17 pts with multiple studies), all with less than 20% BM involvement, using a combined May-Grünwald Giemsa (MGG) (morphology)/FISH approach to determine the plasma cell genotype (target or T-FISH). Cytospin slides were made using 200 l of BM and stained with MGG for morphologic classification on a Duet Image Analyzer (Bioview Ltd., Rehovot, Israel). After identifying and mapping the plasma cells, the slides were destained and hybridized with one of four FISH probe strategies corresponding to the chromosome aberrations listed above. The T-FISH results were correlated with CC and the BM pathology report, which quantified the percentage of plasma cells in the BM aspirate. Target FISH identified MM aberrations in 88/102 (86.3%)samples. The aberrations observed were IGH in 49 samples, -13/del(13q) in 32, hyperdiploidy in 27, hypodiploidy in 6, and del(17p) in 2, with 25 samples showing more than one abnormality. Only 7 samples showed clonal karyotypic aberrations by CC; an additional 3 samples showed a presumed stemline with only one abnormal cell (9.8%). A comparison with % of plasma cells in the BM smears showed T-FISH detected residual disease in all 36 samples with 6 % plasma cells, 7 of 8 hemodilute samples, one smoldering MM sample and 75% (43/57) of the samples with 1-5% plasma cells. Our data indicate target FISH is a quick and reliable genotype/phenotype molecular cytogenetics technique that greatly improves residual disease detection and provides an exceptionally sensitive strategy for investigating low mitotic malignancies such as multiple myeloma.

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Incomplete processing of mutant lamin A in HGPS leads to nuclear abnormalities, which are prevented by farnesyl-transferase inhibition. *M.W. Glynn, T.W. Glover.* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Hutchinson-Gilford progeria syndrome (HGPS) is commonly caused by mutations in codon 608 (G608G) of the *LMNA* gene, which activates a cryptic splice site resulting in the in-frame loss of 150 nucleotides between the cryptic splice site in exon 11 and the beginning of exon 12. The deleted region includes a protein cleavage site that normally removes the C-terminal 15 amino acids, including a CAAX box farnesylation site from lamin A. It has been assumed, but not proven, that the mutant protein remains farnesylated. We have investigated the processing of the C-terminus of the mutant protein, progerin, and have found that it does not undergo this cleavage event and, indeed, remains farnesylated. The retention of the farnesyl group may have numerous consequences as farnesyl groups increase lipophilicity and are involved in membrane association and protein interactions, and is thus likely to be an important factor in the HGPS phenotype. To further investigate this, we studied the effects of farnesylation inhibition on nuclear phenotypes in cells expressing the progerin protein. Expression of an N-terminal GFP-progerin fusion protein in normal fibroblasts caused a high incidence of nuclear abnormalities, as seen in HGPS fibroblasts, and abnormal nuclear distribution of the GFP-progerin in comparison to the localization pattern of GFP-lamin A. Expression of a GFP-lamin A fusion containing a mutation (L647R) that prevents the final cleavage step, causing the protein to remain farnesylated, was found to give rise to identical localization patterns and nuclear abnormalities as in HGPS cells and in normal cells expressing GFP-progerin. Exposure to a farnesyl transferase inhibitor, PD169541, caused redistribution of GFP-lamin A and GFP-progerin localization in normal fibroblasts and a significant improvement in the nuclear morphology of cells expressing GFP-progerin. We also observed a significant improvement in the nuclear morphology of HGPS fibroblasts exposed to this FTI. These results implicate the abnormal farnesylation of progerin in the cellular phenotypes in HGPS cells and suggest that FTIs may represent a therapeutic option for patients with HGPS.

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TGF-induced failure of satellite cell performance and muscle regeneration in mouse models of Marfan syndrome and other myopathic states. *R.D. Cohn[1], J. Pardo[1], B.L. Loeys[1], T.M. Holm[1], D.P. Judge[2], H.C. Dietz[1].* 1) Johns Hopkins Hospital, McKusick-Nathans Institute of Genetic Medicine, Howard Hughes Medical Institute, Baltimore, MD; 2) Division of Cardiology, Baltimore MD.

A large subset of patients with Marfan syndrome (MFS), an autosomal dominant connective tissue disorder caused by mutations in the fibrillin-1 gene, exhibit significant muscle hypoplasia and myopathy. Recent evidence suggests that the pathogenesis of MFS does not simply manifest loss of structural tissue integrity, but rather involves excessive activation of and signaling by the transforming growth factor- (TGF)superfamily of cytokines. TGF has been shown to be a potent inhibitor of terminal differentiation of cultured myoblasts, however the functional impact of TGF signaling on myogenesis in vivo remains obscure. We find for the first time that excessive TGF bioactivity in vivo leads to significant myopathic alterations. Mice homozygous for a missense mutation (C1039G) in fibrillin-1 exhibited significant muscle hypoplasia and muscle hypotrophy in association with nuclear enrichment of pSmad2/3, indicative of excessive TGF signaling. Heterozygote C1039G/+ mice demonstrated myopathic alterations at 3 months of age with a reduction and marked variation in fiber size, and endomysial fibrosis. Identical myopathic changes were observed in patients with MFS. Analysis of skeletal muscle after cardiotoxin injection revealed failed muscle regeneration in C1039G/+ mice secondary to perturbed satellite cell proliferation and differentiation, as evidenced by decreased M-cadherin and myogenin expression, respectively. Systemic administration of TGF neutralizing antibody fully normalized muscle architecture, satellite cell performance and muscle regeneration in response to injury. Additional data suggest that excessive TGF signaling contributes to pathogenesis in the dystrophin-deficient mdx mouse model of Duchenne muscular dystrophy. These data provide the first and compelling evidence for a primary role of dysregulated TGF signaling in myopathic states and impaired muscle repair and thus provide both rationale and motivation to explore the therapeutic utility of TGF antagonism in vivo.

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AT1 blockade rescues multisystem manifestations of Marfan syndrome independent of hemodynamic effects. *J.P. Habashi[1], T. Holm[1], B.L. Loeys[1], D. Bedja[1], E.R. Neptune[1], D.P. Judge[1], H.C. Dietz[1,2].* 1) Johns Hopkins Univ Sch Med, Baltimore, MD; 2) HHMI, Bethesda, MD.

Marfan syndrome (MFS) is an autosomal dominant condition associated with aortic aneurysm and dissection. Murine models indicate that many manifestations, including myxomatous mitral valve disease and impaired pulmonary alveolar septation, are mediated by excess activation and signaling of the TGF family of cytokines. We observed increased TGF signaling in the aortic wall of patients and mouse models of MFS. We hypothesized that treatment with losartan, an angiotensin II type I (AT1) receptor antagonist that can achieve a clinically relevant inhibition of TGF in other disease states, would provide protection from aneurysm both by decreasing hemodynamic stress and attenuating TGF-initiated pathogenetic events. Mice heterozygous for a fibrillin-1 missense mutation (C1039G/+) were randomized at 7 weeks of age to placebo(n=9), losartan(50 mg/kg,n=8) or propranolol(30 mg/kg,n=7) and compared to wild-type. The dose in each treatment arm was titrated to achieve comparable effects on heart rate and blood pressure. Three echo measurements of the aortic root were obtained from a long axis view in systole at 3 time points (baseline, 2 and 4 months of treatment). All analyses were performed blinded to genotype and treatment arm. Aortic growth during the 4 months of treatment was significantly reduced in the losartan (0.060.07mm;$p<0.0001$) and propranolol (0.220.08mm;$p<0.0001$) groups, compared to placebo (0.430.09mm). The growth in the losartan group was less than that seen with propranolol($p<0.004$) and indistinguishable from that seen in wild-type mice(0.090.05mm). Propranolol treatment did not improve elastic matrix architecture while histologic parameters were normalized in losartan-treated mice. Preliminary studies show that administration of TGF-neutralizing antibody provided similar protection ($p<0.02$). We demonstrated that losartan also rescued other aspects of the phenotype including alveolar septation. This represents the first therapy for MFS that derives from interrogation of disease pathogenesis and the first possibility for primary prevention of disease manifestations.

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Dietary methyl donors modify the phenotype of a Rett syndrome mouse model. *P. Moretti[1,2], C.P. Schaaf[1], R. Teague[1], C. Pedroza[5], O. Smith[1], H. Zoghbi[1,3,4].* 1) Dept of Mol and Human Genetics; 2) Neurology; 3) Pediatrics; 4) Howard Hughes Medical Institute Baylor College of Medicine, Houston, TX 77030; 5) University of Texas School of Public Health, Houston, TX 77030.

Mice engineered to express a truncated allele of *MECP2* (*Mecp2*$^{308/Y}$) recapitulate many neurologic features of Rett syndrome (RTT) and provide an excellent model for studying the biological basis of RTT and for testing treatment interventions. MeCP2 is involved in the regulation of methylation-dependent gene expression. Methyl donors such as folate, betaine, methionine, and choline are available as nutritional supplements and have been shown to modify DNA methylation levels in vivo and in vitro and to affect gene expression. We hypothesized that dietary methyl donors modify the neurologic deficits of *Mecp2*$^{308/Y}$ mice by modulating the level of DNA methylation of MeCP2-regulated genes. We performed a controlled randomized trial in which wild-type and mutant animals were exposed prenatally and postnatally to diets containing low or high concentrations of methyl donors. The behavioral and morphologic phenotypes of all animals were studied from weaning until death. *Mecp2*$^{308/Y}$ mice exposed pre- and postnatally to a low methyl donor diet showed significant modification of key phenotypes including decreased motor deterioration and increased life span. The health and behavior of wild-type mice were not influenced by these dietary manipulations. These data indicate that reducing dietary methyl donors improves the neurologic deficits and general health of *Mecp2*$^{308/Y}$ mice. The results raise important questions about the effects of nutrition on the epigenetic regulation of neuronal gene expression. Future work will determine whether dietary methyl donors affect DNA methylation and gene expression in *Mecp2*$^{308/Y}$ mice.

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eQTL mapping in the mammalian eye: Application to the identification of human disease genes. *K.A. Kim[1], T.E. Scheetz[1], R. Swiderski[1], A.R. Philp[1], T.L. Casavant[1], J. Huang[1], E.M. Stone[1,2], V.C. Sheffield[1,2].* 1) University of Iowa, Iowa City, IA; 2) HHMI, Iowa City, IA.

Recent advances in microarray technology and bioinformatics have made it possible to perform experiments that examine the expression of thousands of genes in related individuals and to use these data to identify the chromosomal locations of the genetic elements that are responsible for gene expression variation among individuals. This technique is known as expression quantitative trait locus (eQTL) mapping. In order to gain a broad perspective of mechanisms of gene regulation in the mammalian eye, and to use this perspective to find new genes that cause human eye disease, we performed eQTL mapping in the mammalian eye. Two inbred strains of laboratory rats were crossed and the resultant F_1 animals were used to generate 120 healthy F_2 male animals. The F_2 animals were euthanized at 12 weeks of age, and tissues were harvested. RNA was harvested from the whole eyes and ocular gene expression of each animal was determined using Affymetrix microarrays containing approximately 31,000 probes. DNA was obtained from the liver of each animal and used for genotyping with microsatellite markers across the whole rat genome. Two types of analyses were performed with the data. First, genetic analysis was performed to look for relationships between marker locus and expression. Second, pair-wise analysis of each gene with all other genes was performed to identify correlated variations in gene expression. Of the ~31,000 probes on the array, ~18,000 exhibited sufficient signal and variation in expression among the 120 F_2 animals for reliable analysis. Significant linkage to at least one genetic marker was detected for thousands of probes. In all, more than 4,000 marker-probe linkages were detected at an of 0.001. Both cis- and trans-acting loci were identified. The pair-wise correlated expression analysis make it possible to infer gene expression networks. In a novel application of these data, we used predicted expression networks to prioritize candidate genes within a linkage interval to aid in the identification of a gene that causes a human retinal degeneration syndrome.

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Susceptibility genes for age related maculopathy (ARM) on chromosome 10q26. *J. Jakobsdottir[1], Y.P. Conley[2,3], D.E. Weeks[1,2], T.S. Mah[4], R.E. Ferrell[2], M.B. Gorin[2,4].* 1) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA; 3) Department of Health Promotion and Development, School of Nursing, University of Pittsburgh, PA; 4) UPMC Eye Center, Department of Ophthalmology, School of Medicine, University of Pittsburgh, PA.

We discovered susceptibility genes for ARM of comparable impact to Complement factor H (CFH) which several groups reported during the past year. We performed a SNP genotyping on 594 ARM families and 179 controls on chromosome 10q26. The linkage analysis gave a wide (10 cM) multipoint S_{all} peak and indicated two regions; GRK5 with the highest multipoint S_{all} LOD score (3.07) and PLEKHA1 with two-point S_{all} of 3.34 and the highest two-point HLOD (2.66). Association analysis allowing for related cases showed a strong signal over PLEKHA1 (p-value < 0.00001). We extended this finding by genotyping all known non-synonymous SNPs in the region of three tightly linked genes PLEKHA1, LOC387715 and PRSS11. We also tested for the presence of their proteins in the eye using RT-PCR of human retinal RNA. The follow-up analysis also yielded a strong signal in PLEKHA1 and LOC387715; due to high linkage disequilibrium it is hard to statistically distinguish between those genes. Both PLEKHA1 and LOC387715 are expressed in the eye; PLEKHA1 in the retina and central macula but the expression of LOC387715 appears to be limited to the placenta. The genotype-IBD sharing test suggests that PLEKHA1 (p-value 0.006) is more likely to account for the linkage signal then LOC387715 (p-value 0.018). The association with PLEKHA1/LOC387715 is very similar to the association found between CFH and ARM. In our data PLEKHA1/LOC387715 and CFH account for a dominant odds ratio of 5.0 (3.2-7.9) and 5.3 (3.4-8.4) and attributable risk of 57% and 68%, respectively. Biologically PLEKHA1 and LOC387715 are relevant candidates to contribute to risk of ARM; though more is known about the expression of PLEKHA1 in the eye and its potential role in cellular responses to inflammatory cytokines.

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Mutations in *TCF8* cause posterior polymorphous corneal dystrophy and ectopic expression of epithelial characteristics by corneal endothelial cells. *C.M. Krafchak[1,2], H. Pawar[1], S.E. Moroi[1], A. Sugar[1], P.R. Lichter[1], D.A. Mackey[3,4], S. Mian[1], T. Nairus[1], V. Elner[1], C.A. Downs[1,5], T. Guckian Kijek[1], J.M. Johnson[1], E.H. Trager[1], F. Rozsa[1], M.N. Mandal[1], M.P. Epstein[6], D. Vollrath[7], R. Ayyagari[1], M. Boehnke[8], J.E. Richards[1,2].* 1) Department of Ophthalmology, University of Michigan; 2) Department of Epidemiology, University of Michigan; 3) Department of Ophthalmology, University of Melbourne, Australia; 4) School of Medicine, University of Tasmania, Australia; 5) Department of Otolaryngology, University of Michigan; 6) Department of Human Genetics, Emory University; 7) Department of Genetics, Stanford University; 8) Department of Biostatistics, University of Michigan.

Posterior polymorphous corneal dystrophy (PPCD) is a rare disease involving metaplasia and overgrowth of corneal endothelial cells. In PPCD patients, these cells manifest epithelial morphology and gene expression patterns, produce an aberrant basement membrane and sometimes spread over nearby structures increasing the risk of glaucoma. Within the previously mapped PPCD locus *PPCD3*, we report a heterozygous frameshift mutation in *TCF8*, a gene encoding a two-handed zinc-finger homeodomain transcription factor, that co-segregates with PPCD in the *PPCD3* family, and four different heterozygous nonsense and frameshift mutations in *TCF8* in other probands. Findings of inguinal hernia, hydrocele and possible bone anomalies in affected individuals suggest that those with *TCF8* mutations should be examined for non-ocular anomalies. We detect transcripts of all three identified PPCD genes (*VSX1, COL8A2*, and *TCF8*) in the cornea. We show the presence of a complex (core plus secondary) binding site for TCF8 in the promoter of Alport syndrome gene *COL4A3*, and present immunohistochemical evidence of ectopic expression of COL4A3 in the corneal endothelium of the proband of the *PPCD3* family. Identification of *TCF8* as the *PPCD3* gene provides a valuable tool to study gene regulation events in PPCD pathology and suggests a role for *TCF8* mutations in altering the structure and function of cells lining body cavities other than the anterior chamber of the eye.

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Heterozygous and homozygous mutations in PITX3 in a large Lebanese family with posterior polar cataracts and neurodevelopmental abnormalities. *C. Bidinost[1], M. Matsumoto[2], D. Chung[3], N. Salem[3], K. Zhang[4], D.W. Stockton[5], A. Khoury[3], A. Megarbane[3], B.A. Bejjani[1,6], E.I. Traboulsi[2].* 1) Health Research and Education Center, Washington State University, Spokane; 2) Center for Genetic Eye Diseases, Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH; 3) Medical Genetics Unit, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon; 4) Department of Ophthalmology and Visual Sciences, University of Utah Health Sciences Center, Salt Lake City, UT; 5) Departments of Molecular and Human Genetics, Medicine and Ophthalmology, Baylor College of Medicine, Houston, TX; 6) Sacred Heart Medical Center, Spokane, WA.

The *PITX3* gene that codes for a homeobox bicoid-like transcription factor is responsible for dominant cataract and anterior segment mesenchymal dysgenesis in humans. We performed a linkage study in a large Lebanese family with autosomal-dominant posterior polar cataract (PPC) and found a Lod score of 6.56 (=0.00) to chromosome 10q25. Haplotype and sequencing analysis revealed a *PITX3* mutation that co-segregates with the disease. We also describe two siblings who are homozygous for the *PITX3* mutation who have microphthalmia and significant neurologic disorder characterized by mental retardation, choreiform movements, and paraplegia with increased muscle tone and decreased deep tendon reflexes. This is the first report of homozygous *PITX3* mutations in human. The phenotype in these individuals highlights the role of *PITX3* in ocular and CNS development.

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Genetic association analysis of bitter-taste sensitivity in chimpanzees: convergent evolution at *TAS2R38*. *S. Wooding[1], B. Bufe[2], A. Stone[3], C. Grassi[4], M. Howard[1], D. Dunn[1], W. Meyerhof[2], R. Weiss[1], M. Bamshad[1].* 1) Dept Human Genetics, Univ Utah, SLC, UT; 2) German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; 3) Arizona State University, Tempe, AZ; 4) Southwest Foundation for Biomedical Research, SA, TX.

In humans, sensitivity to the bitter compound, phenylthiocarbamide (PTC), is largely explained by the presence of taster and non-taster alleles at the *TAS2R38* locus. In 1939, Fisher *et al.* reported that chimpanzees also vary in sensitivity to PTC. Like humans, chimpanzees harbor PTC taster and non-taster alleles at roughly 50:50 frequencies. Fisher *et al.* argued that the best explanation for this finding is that humans and chimpanzees share taster and nontaster alleles that originated prior to the human-chimpanzee species divergence. To test this hypothesis, we analyzed patterns of DNA sequence variation in *TAS2R38* in 37 wild-born and 40 captive chimpanzees. Sequences from the wild-born chimpanzees revealed seven haplotypes, which did not differ at any of the nucleotide or amino acid positions distinguishing the seven *TAS2R38* alleles identified to date in humans. Like humans, chimpanzees had two common haplotypes; however, unlike the human taster and non-taster haplotypes, which differ by three amino acids, the chimpanzee common haplotypes differed by a single nucleotide substitution that is predicted to cause a null allele. *In vitro* translation experiments revealed that the putative null allele produces a severely truncated protein that is probably non-functional. Further, expression of the chimpanzee alleles in human embryonic kidney cells revealed that while the chimpanzee wild-type allele had a PTC dose/response curve similar to that of the human taster allele, the null allele showed no response to PTC. Finally, an association analysis in the captive chimpanzees confirmed that the null allele is negatively correlated with PTC sensitivity *in vivo* ($p < 0.001$). These findings suggest that PTC sensitivity in both humans and chimpanzees is determined by taster and non-taster alleles of *TAS2R38*; however, these alleles operate through different molecular mechanisms that are the result of convergent evolution.

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Estimating Age Related Changes in the Causes of Deafness by Sentinel Phenotype Analysis. *W.E. Nance[1], A. Pandya[1], K.S. Arnos[2], B.G. Lim[3], C.C. Morton[4].* 1) Human Genetics, Virginia Commonwealth Univ., Richmond, VA; 2) Gallaudet Univ, Washington, DC; 3) Pediatrix Corp, Sunrise FL; 4) Harvard Medcal School, Boston MA.

A major limitation of existing audiologic newborn hearing screening programs is that they cannot detect late onset prelingual losses if testing is confined to the newborn period. Few if any studies have compared age related changes in the incidence and prevalence of more than one form of deafness in a large population based sample of deaf children identified by newborn hearing screening. We have combined data from England and the US to estimate the overall frequency prelingual hearing loss at birth (186/100,000) and 4 years of age (270/100,000) in the US. Using frequencies of connexin deafness in simplex and multiplex probands with non-syndromic deafness, the proportion of genetic deafness in simplex probands is GS=CS/CM where GS is the proportion, CS is the frequency of connexin deafness in that group and CM the frequency of connexin deafness in multiplex probands. Applying this formula to data from a Nationwide Repository of DNA samples from deaf probands where CS was 0.136, and CM was 0.323, giving 0.421 for Cs. We used this result to estimate that the total proportion of genetic deafness at birth was 68% and 55% at age 4. Using available data we estimated the frequencies of CMV deafness at birth and age 4 as well as Pendred syndrome, non-syndromic EVA, connexin deafness and A1555G mediated aminogycoside ototoxicity. Finally we used race specific frequencies of hearing loss and the racial distribution of births in the US in 2003 to adjust our estimates to reflect the US population. Congenital CMV infections and connexin deafness are the two major causes of deafness at birth in the US, and syndromic and non-syndromic forms of enlarged vestibular aqueduct with hearing loss along with congenital CMV infections are the two major postnatal causes of deafness that begin by the age of four. We estimate that nearly 60% of the infants who develop late onset pre-lingual hearing loss could be identified at birth by screening newborn blood spots for four causes of deafness by molecular genetic techniques.

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Additional Clinical Manifestations in Children with Sensorineural Hearing Loss and Biallelic *GJB2* Mutations: Who Should Be Tested for *GJB2* Mutations? *D.M. Yaeger[1], M.A. Kenna[2,3], A. Frangulov[2], J. McCallum[1], I.D. Krantz[1], H.L. Rehm[4].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia and The University of Pennsylvania, Philadelphia, PA; 2) Department of Otolaryngology, The Childrens Hospital Boston; 3) Department of Otology and Laryngology, Harvard Medical School; 4) Department of Pathology, Brigham and Womens Hospital, Harvard Medical School, Boston, MA.

Sensorineural hearing loss (SNHL) is the most common sensory deficit, occurring in 3 out of every 1000 live births. Worldwide, mutations in the *GJB2* (*gap junction beta 2*) gene, encoding the connexin 26 (Cx26) protein, are the most common genetic cause of hearing loss and are responsible for approximately 30% of all cases of childhood SNHL. *GJB2* mutations are primarily associated with autosomal recessive nonsyndromic bilateral SNHL, although there are rare syndromic forms, most commonly associated with dermatologic manifestations. In general, children with bilateral SNHL and associated non-dermatologic structural or developmental abnormalities are often overlooked as candidates for *GJB2* testing. In a cohort of 139 probands with biallelic *GJB2* mutations, we identified 30 (22%) with additional physical and/or developmental findings. In 21 of these individuals there were sufficient clinical and developmental anomalies to suggest a syndromic etiology for their hearing loss. In the remaining 9 probands, SNHL was seen in combination with a single additional clinical manifestation. Given the high prevalence of *GJB2*-related SNHL, individuals with SNHL and additional findings, which are not definitively explained by a syndromic etiology, should be considered for *GJB2* mutation analysis. While *GJB2* mutations may not be directly responsible for the additional findings, identifying a cause for the SNHL may help to clarify the etiology of the additional findings as well as aid in recurrence risk counseling for families.

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Genomic approaches for pathway identification in regenerating sensory epithelia of the inner ear. *D. Alvarado, K. Powder, D. Hawkins, S. Bashiardes, V. Bhonagiri, R. Veile, M. Warchol, M. Lovett.* Dept Genetics, Washington Univ, St Louis, MO.

Loss of sensory hair cells in the cochlea and vestibular organs of the inner ear is the leading cause of hearing and balance deficits in humans. In mammals, sensory hair cells are formed during embryonic development and if damaged, lack the capacity for regeneration. However, in birds, sensory hair cells can regenerate in response to injury. We have recently completed the first large-scale gene expression profile of regenerating avian hair cells on a custom transcription factor (TF) gene microarray. The sensory epithelia (SE) from avian cochlea and utricle were separately profiled at various recovery time points following damage by either laser or chemical ablation. This study involved hundreds of microarray comparisons and numerous biological samples. Expression profiles for all timecourses were compared to identify common differentially expressed TFs. Many of these identified genes can be placed into known signaling cascades such as the *Ap1* pathway, *Tgf* pathway, sonic hedgehog signaling and the *Pax-Eya-Six-Dach* pathway. To assess the necessity of these TFs for SE proliferation and to identify downstream targets, 20 of these TFs were targeted with siRNAs using a 96-well SE cell proliferation assay. We also employed various pharmacological inhibitors and inducers of defined pathways. Targeted knockdowns of *Pax2*, *JunD* and *Cebpg* significantly inhibited SE proliferation. Inhibition of the Jun kinase (JunK) pathway also inhibited SE proliferation. Additionally, TF gene expression profiles for targeted knockdowns or chemical agents were compared to identify potential epistatic relationships. In this way we have placed many TF genes into previously undescribed interactive relationships. One example of an implicated pathway involves the JunK signaling cascade. Our siRNA and profiling data indicate that *JunD* lies upstream of *Cebpg* and that five additional genes lie downstream of *Cebpg*: *IRX4*, *LRP5*, *TAF-172*, *RARA*, and *ZNF44*. Knockdowns of these genes indicate that *IRX4*, *LRP5* and *TAF-172* all inhibit proliferation and that this cascade may intersect with *Wnt* signaling.

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Prevalence, impact and genetics of slight/mild sensorineural hearing loss in school age children. *H.-H. Dahl, M. Wake, S. Tobin, Z. Poulakis, F. Rickards, B. Cone-Wesson, & the Hearing In Schools Study team.* Murdoch Childrens Res. Inst., Melbourne, AUSTRALIA.

Existing research suggests slight/mild childhood sensorineural hearing loss (SNHL) has important consequences, but this evidence is mainly from clinic or population samples with low (<50%) response rates. Our aim is to determine, from an unbiased population sample, the impact of slight/mild SNHL on language, reading, behaviour and health-related quality of life (HRQoL) in elementary school children and assess the genetic contribution to the slight/mild SNHL. We have completed a cross-sectional observational study of a random cluster sample of 6587 children (85% response; 3367 Year 1, 3214 Year 5) in 89 schools in Melbourne, Australia. All parents reported on childrens HRQoL and behaviour prior to child audiometric assessment. Slight/mild SNHL was defined as low-frequency pure tone average (LPTA) across 0.5, 1, 2 kHz and/or high-frequency PTA (HPTA), across 3, 4, 6 kHz of 16-40 dB HL in the better ear, with air-bone conduction gaps <10dB. Normal hearing was defined as LPTA and HPTA 15dB HL in both ears. Each child with slight/mild SNHL (matched to 2 normally-hearing children on school, year level, age and gender) completed standardised measures of language, reading, phonological skills, non-verbal IQ, and HRQoL. 55 children (0.83%) had slight/mild SNHL; 48 participated in further assessment, along with 96 normally-hearing children. Affected children showed poorer phonological skills, but language, reading, behaviour and HRQoL scores were not significantly different from those of the control group. Four of 48 children with slight/mild SNHL were homozygous for the connexin 26 (Cx26) V37I mutation, 2 were carriers of the V37I mutation. All were of Asian background. Two children in the control group were carriers of known Cx26 mutations (235delC and 35delG). We conclude that the prevalence of slight/mild SNHL is lower than commonly reported, that mutations in the connexin 26 gene is the underlying cause in ~8% of young Australians with a slight/mild SNHL and that the V37I change is a common cause of slight/mild SNHL in children of Asian ethnicity.

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The severity of hearing impairment due to connexin 26 mutations strongly depends on the nature of the mutations: a multi-center study. *G. Van Camp, The connexin 26 genotype phenotype consortium.* Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium.

Hearing impairment (HI) affects 1 in 650 newborns, making it the most common congenital sensory impairment. Despite extraordinary genetic heterogeneity, mutations in one gene, GJB2, are found in up to 50% of patients with autosomal recessive non-syndromic hearing loss. Because of the high frequency of GJB2 mutations, mutation analysis of this gene is widely available as a diagnostic test. We performed a cross-sectional analysis of GJB2 genotype and audiometric data from 1531 persons from 16 different countries with autosomal recessive, mild-to-profound, nonsyndromic hearing impairment. The median age of all participants was 8 years; 90% of persons within the age range of 0-26 years (total age range, 0-70 years). Of the 83 different mutations identified, 47 were classified as non-truncating and 36 as truncating. A total of 177 different genotypes was found, of which 64 were homozygous truncating (T/T), 42 were homozygous non-truncating (NT/NT) and 71 were compound heterozygous truncating / non-truncating (T/NT). The degree of HI associated with biallelic truncating mutations was significantly more severe than the HI associated with biallelic non-truncating mutations ($p < 0.0001$). Forty-seven different genotypes showed less severe HI compared to 35delG homozygotes. Several common mutations (M34T, V37I, L90P) were associated with mild-to-moderate HI (median 25-40 dB; range 10-60 dB for genotypes involving M34T and/or V37I, range 10-110 dB for those involving L90P). Two genotypes (35delG/R143W (median = 105 dB; range = 105-115 dB) and 35delG/del(GJB6-D13S1830) (median = 108 dB; range = 70-120 dB) had significantly more severe HI compared to 35delG homozygotes. The results of this large multicenter study show that the probability of having a severe to profound hearing impaired child strongly depends on the mutations carried by the parents and that the phenotype of future children can be predicted on the basis of these mutations with a certain probability. This has important consequences for genetic counselling for this frequent form of hereditary hearing impairment.

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Pur alpha overexpression suppresses neurodegeneration in the *Drosophila* FXTAS Model. *P. Jin, R. Duan, F. Hassouna, T.C. Rosser, S.T. Warren.* Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA.

Fragile X syndrome premutation carriers have FMR1 alleles containing 55 - 200 CGG repeats. Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder, with neuronal inclusions, that has been recognized in some premutation carriers. We have developed a *Drosophila* model of FXTAS and demonstrated that transcribed premutation repeats alone are sufficient to cause neurodegeneration. It has been proposed that rCGG-Repeat-Binding Proteins (rCGGBPs) may be sequestered from their normal function by binding to lengthy rCGG repeats similar to the myotonic dystrophy mechanism. Using rCGG oligoribonucleotides to capture rCGGBPs from mammalian brain lysate followed by mass spectrometry, we identified Pur alpha as a rCGGBP. In mammals, Pur alpha is a conserved RNA-binding protein that is expressed in neuronal cytoplasm and involved in dendritic mRNA transport. We found that Pur alpha and rCGG repeat interaction is conserved between mammals and *Drosophila*, and is sequence-specific. As our hypothesis predicts, over-expression of Pur alpha in *Drosophila* could suppress rCGG-mediated neurodegeneration in a dose dependent manner. Further, Pur alpha was found to be part of inclusions induced by rCGG repeats in this model. These data support the disease mechanism of the fragile X premutation rCGG repeat sequestering specific rCGGBPs leading to neuronal cell death and suggest that Pur alpha could play a role in the pathogenesis of FXTAS.

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A first generation drug screen for fragile X syndrome. *S. Chang, P. Jin, S.T. Warren.* Human Genetics, Emory University, Atlanta, GA.

Fragile X syndrome is an inherited form of mental retardation that results from the loss of the fragile X mental retardation 1 protein (FMRP). Currently there are only modest palliative treatments for only some of the behavioral aspects of the disorder. FMRP is a translational suppressor of a subset of mRNAs and is found within dendritic spines. Recent data has linked FMRP action with the postsynaptic metabotropic glutamate receptor 5 (mGluR5), whose downstream actions on learning and memory require new protein synthesis. Thus FMRP may act to balance the translational stimulation of mGluR5 activity. Indeed, in the absence of FMRP, the protein encoded by the MAP1B mRNA, a known FMRP ligand is over expressed in neurons. Thus mGluR5 antagonists, such as MPEP, may attenuate this consequence of FMRP loss. We found *Drosophila* with *dFmr1* null mutation exhibits early developmental blockade and lethality before pupae stage on a commercial food source (JAZMIX), which was not apparent with homemade food. Placing ~40 *dFmr1* null embryos in vials of homemade food led to an average of 38 pupae/vial while on JAZMIX only an average of 0.5 pupae/vial (1.3 % false positive). Supplementing JAZMIX with 86 ?M MPEP led to the recovery of 21 pupae/vial. These observations led to the development of a novel drug screen for pharmacological agents that suppress this *dFmr1* null phenotype. A collection of 2,000 FDA-approved, biologically active and structurally diverse compounds was tested. Only 43 drug-treated vials showed evidence of pupae formation (~2.1 %). 9 drugs showed at least 15% pupae recovery or the emergence of *dFmr1* deficient adults. Some of these identified drugs are known to influence the serotonin, dopamine, and mGluR pathways. This screen identified multiple structurally unrelated pharmacological agents, for follow-up studies in *Drosophila* and mice that may modulate the consequence of FMRP loss.

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A conserved function for two *Drosophila* homologs of *Chx10*, a human micropthalmia gene, in the control of proliferation and interneuron differentiation of the visual system. *T. Erclik[1,2], V. Hartenstein[3], H. Lipshitz[1,2], R. McInnes[1,2].* 1) Prog in Dev Biol, Hosp for Sick Children, Toronto; 2) Dept of Mol and Medical Genetics, Univ of Toronto; 3) Dept of Mol, Cell and Dev Biol, UCLA.

Chx10 encodes a homeodomain (HD) and CVC domain protein that is an essential component of a genetic pathway which regulates mammalian eye development. In vertebrates, including human, *Chx10* loss of function is associated with poor proliferation of retinal progenitors, microphthalmia and blindness. Because regulators of eye development are often highly conserved between invertebrates and mammals (e.g.*Pax6*) we are using the powerful genetics of *Drosophila* to facilitate the dissection of the *Chx10* regulatory pathway. We have identified two *Drosophila* homologs of *Chx10*, *dChx1* and *dChx2*, which are >80% identical to *Chx10* in the HD and CVC domains. We report that, remarkably, *dChx1* is expressed in the proliferating progenitors of the fly optic lobe and that a loss of *dChx1* function leads to a great reduction in the size of the optic lobe. This finding suggests that *dChx1* may play a role in the proliferation of optic lobe progenitors of invertebrates that is orthologous to that of *Chx10* in the neuroretinal progenitors of vertebrates. In later stages of *Drosophila* development both *dChx* genes are expressed in the medulla of the mature optic lobe. The medulla contains a class of interneurons that synapse with the photoreceptors required for colour and UV light detection. The expression of the *dChx* genes in the medulla suggests that *dChx*-expressing mature optic lobe interneurons in the fly and *Chx10*-dependent bipolar interneurons in mammals are orthologous populations of visual interneurons. We propose, based on the expression data and mutant phenotypes, that the neuroretina of vertebrates and the optic lobe of flies are evolutionarily conserved structures whose development is regulated by the orthologous homeobox genes, *Chx10* and *dChx1* and *dChx2*. These findings extend the evidence for striking evolutionary conservation of the genetic control of eye development to include the genes that regulate the development of the major interneurons of visual systems.

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Dissection of epistasis in an oligogenic disorder. *J.L. Badano[1], C.C. Leitch[1], S.J. Ansley[1], H. May-Simera[2], R.A. Lewis[3], P.L. Beales[2], H.C. Dietz[1,4], S. Fisher[1,5], N. Katsanis[1,6].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Molecular Medicine Unit, Institute of Child Health, University College London, London WC1N 1EH, UK; 3) Departments of Molecular and Human Genetics, Ophthalmology, Pediatrics, and Medicine, Baylor College of Medicine, Houston, TX; 4) Howard Hughes Medical Institute; 5) Department of Cell Biology, Johns Hopkins University, Baltimore, MD; 6) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

Epistatic interactions are though to play a significant role in phenotypic variation, yet the genetic dissection of such phenomena remains challenging. Through a combination of computational and experimental approaches, we have identified a novel locus, *BBSIP1*, which contributes epistatic alleles to Bardet-Biedl syndrome (BBS), a pleiotropic, oligogenic disorder. *BBSIP1* encodes a pericentriolar protein that interacts and co-localizes with the BBS proteins. Sequencing of two independent BBS cohorts revealed a significant enrichment of a heterozygous 430T mutation in patients and TDT analysis showed strong over-transmission of this variant. This mutation results in the utilization of a cryptic splice acceptor due to the aberrant potentiation of an exonic splice enhancer, deletion of five nucleotides and a moderate reduction of steady-state *BBSIP1* mRNA. Finally, recapitulation of the human genotypes in zebrafish shows that modest suppression of *bbsIP1* in early embryos exerts an epistatic effect on the expressivity of the developmental phenotype of BBS morphants. Our data demonstrate how the combined use of biochemical, genetic and in vivo tools can facilitate the dissection of epistatic phenomena and enhance our appreciation of the genetic basis of phenotypic variability.

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Bardet-Biedl Syndrome genes are involved in a common pathway affecting intracellular trafficking and cilia maintenance. *M. Tayeh[1, 3], H.-J Yen[1, 3], R. Mullins[4], C. Searby[1, 3], T. Westfal[2], E. Stone[3, 4], D. Slusarski[2], V. Sheffield[1,3].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Biology, Univ Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute, Bethesda, MD; 4) Dept Ophthalmology, Univ Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is characterized by obesity, retinopathy, polydactyly, cognitive impairment, renal and cardiac anomalies, hypertension and diabetes. The eight known BBS genes do belong to the same functional category; yet mutation of these genes results in a similar phenotype. While the precise functions of the BBS proteins are unknown, data support a role in ciliated organisms and study of individual BBS proteins indicate involvement in basal body biology. To gain insight into the biological processes controlled by BBS genes, we embarked on studies of six BBS orthologues from zebrafish. Knockdown of zebrafish bbs2, 4, 5, 6, 7 or 8, using antisense morpholinos (MO), results in a specific defect in a non-ciliated cell type altering retrograde organelle (melanosome) transport and a progressive loss of ciliated cells within a developmental structure known as Kupffers vesicle (KV). KV is a ciliated organ thought to play a role in left-right patterning. Our data demonstrate abnormal KVs in MO-treated embryos and subsequent alterations to organ laterality, including heart and brain laterality defects.To confirm the specificity of the generated phenotypes, we rescued with in vitro transcribed wild-type RNA. We further demonstrate that RNA with putative human BBS mutations do not rescue the phenotypes. We also embarked on using zebrafish as a model system to investigate genetic interactions between the zebrafish bbs2, bbs6 and bbs7 genes using co-injection of combinations of MOs targeting the zebrafish bbs genes. Double knockdown data show an additive effect of the phenotype severity. In summary, our data demonstrating overlapping cellular phenotypes of the individual BBS knockdowns and additive effects of the double knockdown suggest that mutated BBS genes affect a common pathway involved in general organelle trafficking and cilia maintenance and that BBS genes can potentially modify each others phenotype.

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Organ laterality: cilia direct a wave of asymmetric gene expression that establishes the left and right sides of developing organs. *J.D. Amack, X.H. Wang, H.J. Yost.* Huntsman Cancer Inst, Univ Utah, Salt Lake City, UT.

Reversed organ laterality (*situs inversus*) in patients affected with a subtype of primary ciliary dyskinesia, known as Kartagener syndrome, provides a link between cilia function and organ laterality that has been recognized for many years. Recently, work in mouse and zebrafish embryos has established a model that explains the role of cilia in left-right (LR) patterning of asymmetric organs such as the heart, brain and gastro-intestinal tract. In this model, motile cilia generate a leftward fluid flow that is required for asymmetric expression of a conserved battery of genes on the left side of the embryo, including members of the *nodal* and *pitx2* gene families. In zebrafish embryos, these specialized cilia reside in a transient organ called Kupffers vesicle (KV). We have previously demonstrated that KV cilia create a directional fluid flow, and that perturbation of KV form or function alters LR patterning. Here, we show that expression of the zebrafish nodal family member *southpaw* (*spaw*), which is initiated in left lateral plate mesoderm (LPM) lateral to KV in the tail, progresses as a dynamic posterior-to-anterior (P-A) wave to generate molecular asymmetry in the developing heart, brain and gut. By analyzing mutant embryos in which KV is abnormal, we show that normal KV morphology is not necessary for initiation of *spaw* in LPM, but is required to direct this initiation to the left side. Spatio-temporal analysis of *spaw* expression using *myod* as a somite stage marker indicated that the anterior boundary of *spaw* moved anteriorly at a speed of 3 somite lengths per somite generation time between the 12-20 somite stages. *spaw* expression then rapidly diminished in a P-A fashion between the 20-24 somite stages. Further expression analyses revealed that two additional genes involved in LR patterning*pitx2* and *lefty1*show a similar P-A wave that appears coordinated with *spaw*. Cell labeling experiments indicate the P-A wave is not a result of cell migration, suggesting cell-to-cell signaling as a likely mechanism. These results suggest that a wave of asymmetric gene expression transfers LR pattern information generated by cilia to developing organ primordia.

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Fishing for functional DNA: Efficient functional examination of noncoding sequences in transgenic zebrafish. *A. McCallion[1,2], S. Fisher[1], E.A. Grice[1], R. Vinton[1].* 1) McKusick-Nathans Inst Gen Med, Johns Hopkins Univ, Baltimore, MD; 2) Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Evolutionary sequence conservation is recognized as a reliable indicator of functional sequences (coding and noncoding). With accessible transparent embryos, the zebrafish Danio rerio is an attractive model system to test noncoding putative regulatory elements during development. However, significant problems with mosaicism have hindered the use of transgenic zebrafish to assay for functional noncoding sequences. We have overcome these limitations with a novel transgenic strategy, using the recently described Tol2 transposon. Tol2-based vectors provide a high frequency of plasmid integration and 5 fold higher germline transmission frequencies (mean>=25%) than established protocols. This permits rapid examination of the ability of non-coding sequences to direct tissue-specific GFP expression in live G0 zebrafish. We are systematically testing the hypothesis that conserved non-coding sequences will frequently play a role in regulating expression of their neighboring genes. We have begun by analyzing conserved sequences at the *RET* gene encoding a critical receptor tyrosine kinase in the genesis of the multiple organ systems, and one in which regulatory mutations have already been implicated in human disease (Hirschsprung Disease). To date we have studied 27 amplicons containing 42 conserved non-coding sequence elements, associated with the human and zebrafish *RET* genes, examining their ability to drive consistent reporter expression in live G0 embryos. The majority (24/27) recapitulate endogenous RET expression (CNS, PNS, kidney), including amplicons encompassing >=22 human sequences that display no overt sequence conservation with the teleost genome (>=100bp, >=75%). We are extending these analyses to other critical genes in nervous system development and osteogenesis. This system will facilitate the rapid functional analysis of conserved sequences from multiple vertebrate genomes, and will aid in the refinement of computational approaches to identify fundamental aspects of sequence conservation.

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Genetic backgrounds susceptible to genomic deletions: *Alu*-mediated mutations of BRCA1 as a model. *K.M. Chisholm[1], P.L. Welcsh[2], K.P. Freese[2], M.-C. King[1,2].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA.

At the BRCA1 gene, large genomic deletions frequently occur as somatic loss of heterozygosity in breast tumors and as inherited mutations in families. These genomic deletions appear to be the result of aberrant homologous recombination between *Alu* elements, which are densely packed at the BRCA1 genomic locus. My hypothesis is that loss of function of a still unknown mutator gene(s) leads to increased frequency of homologous recombination and thereby increased frequency of large genomic deletions at susceptible loci. The purpose of my project is to identify such mutator gene(s) using BRCA1 as a model. I have created a construct incorporating BRCA1 exon 17 and flanking *Alu* sequences; these *Alu* repeats are often rearranged in inherited breast tumors. I have inserted this construct, as well as a URA3 selection gene, into a yeast transformation plasmid. This plasmid has successfully been screened for genomic deletions in 99% of the Saccharomyces Genome Project collection of yeast strains comprised of all viable individual gene knockouts. The resulting 80 putative mutator strains include kinases, DNA repair proteins, and proteins involved in meiotic recombination between homologous chromatids. The assay also identified mutator genes found to cause gross chromosomal rearrangements in previous studies, as well as genes such as *msh5* and *pms1*, suggesting that the screen was effective. The potential mutator strains are being analyzed to determine the kinds of mutations they cause and at what rate. Human homologs have been identified thus far for 47 of the 80 genes; mutations in these human genes will be screened for in high-risk breast cancer families who are wildtype for all known breast cancer genes and in sporadic breast tumors. Though the primary aim of this research proposal is to identify mutator genes involved in breast carcinogenesis, these same genes may be involved in other cancers as well. Thus, identifying mutator genes may not only help in understanding breast cancer, but also other cancers.

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Yeast model system reveals the mechanism of palindrome-mediated chromosomal translocation. *T. Ohye[1], H. Inagaki[1], H. Kogo[1], B.S. Emanuel[2], H. Kurahashi[1].* 1) Div Molecular Genetics, Fujita Health Univ, Toyoake Aichi, Japan; 2) Div Human Genetics, Children'fs Hosp Philadelphia, Philadelphia PA.

The constitutional t(11;22)(q23;q11) is the most frequently occurring non-Robertsonian translocation in humans. The breakpoints of the t(11;22) were identified within palindromic AT-rich repeats (PATRRs) on chromosomes 11 (PATRR11) and 22 (PATRR22), suggesting that cruciform structures mediate double-strand-breaks (DSBs) leading to this recurrent translocation. The breakpoints of a variety of translocations involving PATRR22 have been reported, which demonstrated similar palindromic sequences on partner chromosomes, such as 17q11, 4q35.1, and 1p21.2. To investigate why the partner of a PATRR-mediated translocation is preferentially a PATRR located on another chromosome, we analyzed the mechanism of PATRR-mediated chromosomal translocation using yeast model. Previously, we created a diploid yeast harboring the 445bp PATRR11 at the TRP1 locus on chromosome IV and the 595bp PATRR22 at the LEU2 locus on chromosome III. We successfully recapitulated PATRR-mediated chromosomal translocations. We introduced a recognition sequence for a restriction enzyme, I-SceI, into the yeast genome instead of the PATRR22. This would induce mandatory 3'f-overhang DSBs through transient expression of I-SceI. However, we did not detect any translocation-specific PCR products between the PATRR and the I-SceI recognition site. Next, we determined the 5'f and 3'f end of DSBs at the PATRR by ligation-mediated PCR and tailing-mediated PCR, respectively. The majority of the 3'f ends of both proximal and distal sides of the DSB appear to cluster at the starting point of the PATRR. However, the 5'f ends of both sides are distributed throughout the PATRR, and further extend beyond the center of the palindrome. Finally, sequence analysis of the junction fragments indicates that the translocations always occur at a region with microhomology between PATRR11 and PATRR22, and that some breakpoints traverse beyond the tip of the PATRR. We propose that the unusual 5'f overhang DSBs at both PATRRs followed by single-strand annealing is the mechanism that mediates the recurrent chromosomal rearrangement.

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Human inversion polymorphisms identified through studies of inversion events in recent primate evolution. *L. Feuk, J. MacDonald, A.R. Carson, T. Tang, S.W. Scherer.* Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada.

The aim of this study was to investigate inversions that have occurred during recent primate evolution, including the most recent inversions that are polymorphic in the human population. We initially identified all inverted sequences from the net alignments between the human and chimpanzee genome assemblies (human NCBI Build 35 and chimpanzee NCBI Build 1). Segmental duplications were identified as the major source of non-syntenic alignments and these regions were therefore excluded. After this filtering, a total of 2868 regions larger than 20bp remained. Thirteen of the inversions between human and chimpanzee are larger than 100kb and not previously described in literature. We chose 30 regions for experimental validation. Using fluorescence in situ hybridization or PCR, 23 regions were confirmed to be inverted between human and chimpanzee. Using gorilla as an outgroup, 14 inversions were shown to have arisen in the human lineage. Upon examination of 20 human control samples, two of these regions were found to be polymorphic inversions. Three individuals were heterozygous for a 700kb inversion region flanked by segmental duplications. The second region found to be polymorphic involves an 18kb region, of which 13kb are inverted and 5kb are deleted. The allele frequency of the inversion/deletion allele is 70% in control individuals. These results indicate that inversions have been a common rearrangement in recent primate evolution. Encouraged by these findings, we have expanded our analysis to include an alignment between the human R27C assembly from Celera and NCBI Build 35 in order to identify further candidate polymorphic regions. A total of 590 intra-scaffold regions of inverted orientation were identified from this alignment. These regions are now being evaluated experimentally and preliminary results indicate that this dataset includes both assembly errors and inversion polymorphisms.

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A fine-scale map of recombination rates and hotspots across the human genome. *G. McVean, S. Myers, C. Freeman, L. Bottolo, P. Donnelly.* Department of Statistics, University of Oxford, United Kingdom.

Genetic maps, which document the way in which recombination rates change with physical position over a genome, are an essential tool for many genetic analyses. Here we present a high resolution genetic map of the human genome, based on statistical analyses of genetic variation data. The new map details extensive recombination rate variation over kilobase scales, around three orders of magnitude finer than existing pedigree-based human genetic maps, and will facilitate more powerful genetic association and fine-mapping studies, in addition to population genomic analyses. We identify more than 25,000 recombination hotspots, an average of one per 108kb, and no regions larger than 200kb that are devoid of recombination. Recombination rate variation over centimorgan scales, such as the marked differences between telomeres and centromeres, results from changes in both the density and intensity of hotspots. While much of the rate variation over these large scales can be explained by DNA sequence and structural features, the same features fail to predict the precise location of individual hotspots. We also show that recombination rates over large scales appear to be slow-evolving, in contrast to recent evidence that the local recombination landscape is evolving rapidly. This suggests a two stage model for recombination in which hotspots are stochastic features within a framework in which large-scale rates are constrained.

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Genome-wide distribution of genes and sequence features in the context of linkage disequilibrium values. *A.V. Smith[1,2], D.J. Thomas[3], H.M. Munro[4], G.R. Abecasis[4].* 1) Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY; 2) Genthor ehf., Gardastraeti 11, Reykjavik, Iceland; 3) Biomolecular Engineering Department, Baskin School of Engineering, University of California at Santa Cruz, 1156 High Street, Santa Cruz, CA; 4) Center for Statistical Genetics, School of Public Health, University of Michigan, 1420 Washington Heights, Ann Arbor, MI.

The current availability of large-scale datasets providing information on linkage disequilibrium for >1 million markers will greatly aid in the design and interpretation of genome-wide association studies and facilitate the identification of alleles underlying susceptibility to complex disease. In addition to facilitating disease association studies, these datasets also provide us with the best opportunity yet to explore the relationship between local sequence variation and patterns of linkage disequilibrium (LD). Using data from the International HapMap Consortium, we characterize the relationship between local sequence variation and patterns of LD in the genome in three different populations. Our results show remarkable consistency across populations and suggest that GC content, DNA polymorphism, and repeat content are strongly associated with the local extent of LD. We find that genes and coding sequences are enriched in regions of very high and very low disequilibrium, compared to the rest of the genome. Interestingly, when we used the Gene Ontology database to aggregate genes according to their functional roles, we found different types of genes locate preferentially to the high LD and low LD regions. Genes contained involved in the cell cycle control and DNA metabolism are more often found in regions of high LD. In contrast, genes found with a role in immune responses are more often found in regions of lower LD, as they might benefit from increased allele and haplotype diversity. These results suggest that genes within in regions of lower LD might be involved in pathways that benefit from greater diversity, whereas genes in regions of high LD might be involved in more evolutionary conserved processes.

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Annotation of the protein-coding genes in the ENCODE regions. *A. Reymond[1,2], F. Denoeud[3], J. Harrow[4], C. Ucla[2], E. Eyras[3], C. Wyss[2], J. Lagarde[3], E. Birney[5], T. Hubbard[4], R. Guigo[3], S.E. Antonarakis[2], ENCODE consortium members.* 1) Center of Integrative Genomics, University Lausanne, Switzerland; 2) Genetic Medicine, University of Geneva, Switzerland; 3) IMIM, Barcelona, Spain; 4) Wellcome Trust Sanger Institute, Cambridge, UK; 5) EBI, Cambridge, UK.

The aim of the ENCODE project (http://genome.gov/10005107) is to identify all the functional elements of the human genome. In its pilot phase, ENCODE focuses on 44 regions of the human genome corresponding to 1% of its sequence. Within this pilot phase, the GENCODE subgroup (http://genome.imim.es/gencode/) has produced a detailed reference annotation. First, all the 426 known genes mapping to the ENCODE regions were subjected to 5 RACE in 12 tissues. These experiments showed that many known genes are not fully annotated at their 5 as 10-15 % of the RACEs products exhibit yet undescribed exons at their 5end. Second, the genes categorized as putative or novel, were experimentally verified by RT-PCR in 24 different human adult and fetal tissues. Full-length structure of the confirmed genes was obtained by bi-directional RACE. Third, all the 1255 computational predictions from ab initio and EST-based programs that fell outside of our reference annotation were tested experimentally. Only about 3% (32/1215) of them could be verified, suggesting that not many standard protein coding genes are missed from the current set of human genes. The reference annotation of the 44 regions was released in June, 2005. The annotation revealed the complex transcript structure of the human genome with multiple overlapping transcription units through the genome, challenging the traditional view of genes encoded as discrete entities. Alternative splicing is widespread: all multi-exonic genes have more than one splice form. The second phase of the GENCODE project will target specifically those genes and exonic variants likely to be underrepresented in the current catalog of human genes: short and intronless genes, genes undergoing non-canonical splicing, selenoprotein genes, genes with unusual codon composition that may be expressed at very low levels or with a very restricted pattern, and human specific genes, among others.

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Islands of euchromatic-like sequence and expressed genes within the heterochromatic regions: lessons from the initial sequence analysis of 21p. *P. Prandini[1], R. Lyle[1], K. Osoegawa[2], B. ten Hallers[2], S. Humphray[4], B. Zhu[2], E. Eyras[2], R. Castelo[3], C. Bird[4], M. Cruts[5], S. Dahoun[1], X. She[6], C. Van Broeckhoven[5], E. Eichler[6], S.Y Wang[7], R. Guigo[3], J. Rogers[4], P. de Jong[2], A. Reymond[1], S.E. Antonarakis[1].* 1) Univ. Geneva, CH; 2) Children's Hospital Oakland Res. Inst, USA; 3) Ctr Genomic Regulation, Spain; 4) Sanger Institute, Cambridge, UK; 5) Univ. Antwerp, Belgium; 6) Univ. Washington, Seattle, USA; 7) Shangai Genome Ctr, China.

Although the sequence of the euchromatic portion of the human genome is essentially complete, the heterochromatic regions (5-10% of the genome) remain to be sequenced. These regions include the short arms of the acrocentric chromosomes, which are involved in many translocations associated with disease phenotypes. Chromosome 21 has special significance because of its involvement in Down Syndrome and we have thus initiated an international collaboration to determine the sequence of 21p. We have constructed a BAC library from the human-mouse somatic hybrid cell line WAV17, which contains sequence from a single Hsa21. We present here data on 1.3Mb of new sequence from 21p (estimated to be 10-15% of the total) generated from 8 BAC clones isolated with STSs mapping to 21p. Unexpectedly, 21p contains islands of sequence showing euchromatic-like features with an interspersed repeat content very similar to the 21q. Gene predictions by EST and in silico based methods identified 29 gene models, 30% of which were validated by RT-PCR in 24 human tissues. In addition, we mapped the 5 ends of each transcript by RACE. Thus far we have identified an expressed sequence approximately every 100kb, each of which are highly conserved in the chimp genome. Analysis of these transcripts from different individuals and monochromosomal cell hybrids shows extensive variability and suggests that multiple inter- and intrachromosomal copies exist. Quantification of their copy number by qPCR indicates that many are present in 20-50 copies in the human genome. This study is an important step in the characterization of these unexplored regions of the genome and toward the real completion of the human genome project.

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A minority of Conserved Non-Genic sequences (CNGs) of chromosome 21q show properties of transcription regulators. *C. Attanasio[1], J.A. Stamatoyannopoulos[2], R. Lyle[1], M. Dorschner[2], R. Humbert[2], E.T. Dermitzakis[3], A. Reymond[1,4], S.E. Antonarakis[1].* 1) Dept Genetic Medicine & Development, University of Geneva, Switzerland; 2) Dept Molecular Biology, Regulome, Seattle, USA; 3) The Wellcome Trust Sanger Institute, Cambridge, UK; 4) Center for Integrative Genomics, University of Lausanne, Switzerland.

Comparative genome analysis across mammals has revealed a large number of highly conserved non-genic sequences (CNGs). We had studied CNGs from 21q and found that their evolutionary features strongly suggested functional importance. However the function of the majority of these sequences is unknown. A possible role of a subset of CNGs is transcriptional regulation. Indeed sequences involved in transcription regulation are often conserved. In vitro luciferase expression assays on 100 CNGs versus 20 control sequences (single-copy, non-conserved, same GC content), was carried out in two different human cell lines (293T & Huh7). Only 9% of these CNGs displayed a 2-fold increase in luciferase activity compatible with enhancer function. Overall, however, there was no statistically significant difference between CNGs and control sequences in this assay. Since DNaseI hypersensitivity (HS) is a known property of classical cis-regulatory elements (promoters, enhancers, insulators, silencers), we used this characteristic to explore the chromatin structure of a set of 192 CNGs in 8 different human cell types representing derivatives of the 3 embryonic layers. 13% of the CNGs were HS in one or more cell lines. Interestingly, the majority of HS CNGs displayed a cell-type specific DNaseI hypersensitivity, consistent with a tissue-specific role. Finally, as a functional link between the assays used here and gene expression variation in vivo, we assayed expression of 128 genes on Hsa21 by qRT-PCR in 7 cell lines. We are currently investigating a potential correlation between the presence / absence of a cell-type specific HS CNG with cell-specific gene expression. Taken together, the results indicate that only a minority of CNGs have properties of transcriptional regulators.

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Multiple Mammalian Genomes. *R. Gibbs, J. McPherson, J. Belmont, D. Wheeler, G. Weinstock, I. Yakub, A. Goldman, C. Davis, D. Burgess, J. Noebels.* Hum Genome Seq Ctr, Baylor Col Medicine, Houston, TX.

Multiple mammalian genomes have been sequenced and analyzed and we are now turning our efforts back to the understanding of the biology of individual diseases by discovery of specific alleles that underlie different human disease conditions. To accomplish this we established a pipeline at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) that allows sequencing of PCR products from thousands of exons and samples, thereby simultaneously screening large numbers of candidate disease genes in several affected populations for both putative functional mutations and associated polymorphisms. The pipeline is also being used to re-sequence several genes and regions, including behavioral disorder candidates, and West Nile Virus susceptibility regions. We are also searching for causative mutations in patients with Idiopathic Generalized Epilepsy by systematic re-sequencing of the exons and 5 regions of all ~300 ion channel genes. Over 100 patient samples have been collected and a total of 500 will be banked. In the first few genes and patients we are already finding multiple coding changes that may be associated with disease. To attribute functional significance to the changes we are both studying marker frequencies in matched controls and systematically reconstructing the mutant proteins in order to perform patch clamp assays. The ion channels are likely involved in other neurobehavioral disorders including autism, and the survey will be expanded into these by the third quarter of 2005.

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Overcoming a Technical Hurdle in Identifying Variation and Mutations in Large Genomic Regions: Direct Genomic Selection (DiGS). *R. Veile, A.M. Bowcock, M. Lovett.* Division of Human Genetics, Department of Genetics, Washington University School of Medicine, St Louis, MO.

The search for DNA sequence variants that underly many genetic diseases increasingly requires the resequencing of very large genomic regions, including intragenic and intergenic intervals. We recently describe modifications to direct selection that provide a rapid route to the targeted resequencing of large genomic regions (>100kb) and the identification of novel variants, in patient genomic DNAs. This method employs biotinylated BAC DNAs in a hybridization-selection scheme with PCR-linkered total genomic DNAs from patients. This resulted in enrichments of 10,000-fold, in which 50% of the sequence-ready clones were from the targeted region. In a resequencing project of psoriasis patient DNAs covering 150kb of the human RAPTOR gene, we detected 69 known SNPs and ~100 new variants (including several small insertion/deletions). We have now implemented this scheme on several additional genomic intervals and have made the following four modifications: (1) the use of larger BAC contigs of >500kb; (2) we have addressed the issue of contig coverage and completion in the final resequenced interval, by employing random shearing, repair and linker ligation of the target genomic DNAs to derive more even clone coverage; (3) to reduce the number of false positive SNPs (introduced by Pfu polymerase in the large number of PCR cycles), we have optimized the number of PCR cycles and cloning efficiencies, and; (4) to adapt this scheme for cross-species genomic selections we have used a chicken BAC (from which this reference avian genome was derived) to select the orthologous contigs from 50 different diverse bird species. This type of cross-species genomic selection should speed up targeted sequencing (and comparative genomics) of many different species for which BAC libraries and genomic DNA sequences are currently unavailable.

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Genome-wide Mapping of DNaseI Hypersensitive Sites by Massively Parallel Signature Sequencing (MPSS) and DNase-chip Technologies. *G. Crawford[1], S. Davis[1], P. Scacheri[1], I. Holt[1], J. Whittle[1], E. Margulies[1], B. Webb[1], J. Bernat[2], D. Ginsburg[2], S. Luo[3], D. Zhou[3], T. Vasicek[3], R. Green[4], T. Wolfsberg[1], F. Collins[1].* 1) NHGRI, NIH, Bethesda, MD; 2) University of Michigan, Ann Arbor, MI; 3) Solexa, Inc., Hayward, CA; 4) Nimblegen Systems, Inc., Madison, WI.

A major goal in genomics is to understand how genes are regulated in different tissues, stages of development, diseases, and species. Mapping DNaseI hypersensitive (HS) sites within nuclear chromatin is a powerful and well-established method of identifying many different types of regulatory elements, but in the past has been limited to analysis of single loci. We have developed two methods to identify DNase HS sites on a genome-wide scale. First, we report high throughput analysis, using massively parallel signature sequencing (MPSS), of 500,000 tags from DNase libraries generated from quiescent and activated human CD4+ T cells. Of the tags that uniquely map to the genome, we identified 26,000 clusters of sequences that group within close proximity to each other. Using a real-time PCR strategy, we determined that the majority of these clusters represent valid DNase HS sites. Approximately 80% of these DNase HS sites uniquely map within one or more annotated regions of the genome believed to contain regulatory elements, including regions 2kb upstream of genes, CpG islands, and highly conserved sequences. Genes with a DNase HS site nearby had higher expression levels than average, indicating that many of these sites correlate with activation of transcription. Interestingly, 10% of the DNase HS sites are exclusive to lymphocytes, suggesting that this procedure can identify gene regulatory elements that control cell type specificity. We have also developed a second technology, called DNase-chip, which hybridizes genome-wide captured DNase HS sites to custom tiled oligonucleotide microarrays. This method is highly reproducible and can be used to identify DNase HS sites around a single gene or across the entire genome. These strategies, which can be applied to any cell line or tissue from any sequenced organism, will enable a better understanding of how chromatin structure dictates cell function and fate.

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A Survey of Copy-Number Polymorphisms using a set of twenty high density SNP Genotyping Arrays. *H. Matsuzaki, X. Di, G. Liu, H. Loi, J. Law, T. Berntsen, M. Chadha, B. Nguyen, G. Yang, T. Webster, P.S. Walsh, R. Mei.*
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Recently, hundreds of Copy-Number Polymorphisms (CNPs) have been observed in normal individuals. CNP regions are on the order of hundreds of kilobases and are distributed across the genome, often in close proximity to segmental duplications. The vast majority of CNPs were identified in comparative genome hybridization studies using DNA microarrays. Here, we used a set of twenty high density oligonucleotide arrays originally designed to genotype SNPs, to instead identify CNP regions. The set of arrays contains probes for ~ 2.2 million SNP sites, and has a resolution of one probe set every ~1.5 kb across the genome. Hybridization intensities from probes for the two SNP alleles were combined, and then normalized across individuals using Quantile Normalization. We assayed 48 unrelated individuals belonging to four ethnic groups. Probe intensities from the 48 individuals were clustered in sliding windows of 25 SNPs; and regions where adjacent windows showed significant clustering were identified as possible CNP regions. The regions identified in our survey totaled ~ 4% of the genome, and overlapped with 39% (137 of 352) of CNPs currently reported in the Database of Genomic Variants (The Centre for Applied Genomics). The known CNPs have an average length of ~ 200 kb, while the regions identified in our survey tended to be shorter, with an average length of ~50 kb. Consequently, in many of the overlapping regions there was a many-to-one relationship between our regions and the known CNP regions. The identified regions, on average, spanned ~ 21.5 SNP sites. For ~ 600,000 of the ~ 2.2 million SNP sites surveyed, we determined SNP genotypes in the 48 individuals, which enabled us to investigate possible associations between genotypes/haplotypes and individual variations in the CNP regions.

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Q62R Substitution in KLF11 Gene Is Associated with Type 2 Diabetes and Related Traits in Pima Indians. *L. Ma, R.L. Hanson, L.N. Que, P.W. Franks, J. Baer, A.M. Infante, S. Kobes, C. Bogardus, L.J. Baier.* NIDDK/PECRB, NIH, Phoenix, AZ.

The TGF-¦Â signal pathway is important for normal pancreatic development. Since KLF11 is a TGF-¦Â inducible transcription factor, we analyzed this gene as a candidate gene for type 2 diabetes in Pima Indians, who have the highest reported prevalence of this disease in the world. Sequencing of the 4 exons, exon-intron boundary regions, 5' and 3' untranslated regions, and 2kb of the 5' (putative promoter) region of KLF11 in 24 non-first-degree related Pimas identified 9 variants, one of which was a non-synonymous amino acid substitutions (Q62R). These variants and other SNPs available in public databases were genotyped for association analysis in 1300 Pima Indians from 332 nuclear families. The Q62R was associated with young onset type 2 diabetes (onset-age under 45 years) (P=0.017, odds ratio=2.44, CI=1.17-5.08, additive model; P=0.005, odds ratio=3.05, CI=1.40-6.64, recessive model defined as QQ vs QR+RR) after adjusting for sex, nuclear family membership and Pima heritage. The risk allele Q had a frequency of 0.956. When analyzed using a within-family model in the same group of subjects, the Q62R was again associated with young onset type 2 diabetes (P=0.023, odds ratio=4.19, CI=1.22-14.45, additive model; P=0.005, odds ratio=6.49, CI=1.74-24.2, recessive model) after adjusting for sex, nuclear family membership and Pima heritage. In a subgroup of 261 non-diabetic full-heritage Pimas who have undergone detailed metabolic testing including measurements of body composition, oral glucose tolerance (OGTT), hyperinsulinemic-euglycemic clamp, and indirect calorimetry, the risk allele Q was associated with a higher plasma glucose concentration at 0', 30', and 120' (P=0.038-0.002; recessive model), lower plasma insulin concentration at 30' (P=0.0005, recessive model) during OGTT, and lower post-absorptive glucose oxidation rate (P=0.01, recessive model) after adjusting for age, sex, percentage of body fat, and family membership. These findings suggest that the Q allele at Q62R is associated with increased risk of type 2 diabetes as a result of impaired insulin secretion in Pimas.

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Mutations in NOTCH2 cuase Alagille syndrome (a heterogenous disorder). *D. Warthen, R. McDaniell, A. Pai, J.J.D. Morrissette, B.M. Kamath, D.A. Piccoli, I.D. Krantz, N.B. Spinner.* Dept Path and Lab Med and Human Gen, Abramson Res Bldg, Children's Hosp, Philadelphia, PA.

Alagille Syndrome (AGS) is a dominant, multi-system disorder consisting of cholestasis, cardiac defects, butterfly vertebrae, posterior embryotoxon, and characteristic facies. We find that >90% of patients with a diagnosis of AGS have mutations in Jagged1 (JAG1), a ligand in the developmentally critical Notch Signaling Pathway. There are 4 human Notch receptors (Notch 1,2,3,4) that can potentially interact with JAG1. The mouse model for a heterozygous Jag1 mutation is not like AGS, but encompasses eye defects only. However, the Jag1/Notch2 double heterozygote has the liver, cardiac, eye, and renal defects found in patients with AGS, suggesting that Notch2 is the receptor important in these tissues. We studied 248 patients with a diagnosis of AGS and found JAG1 mutations in 227 (91.5%). Twenty-one patients did not have mutations, and we screened them for mutations in NOTCH2 by sequencing all 34 exons. We identified 2 mutations predicted to impact the function of the NOTCH2 protein. In both cases, the probands inherited a single mutation from their affected mother, indicating an autosomal-dominant form of inheritance. The probands have classic features of this disease. The first mutation is an AG>AA alteration at the splice acceptor site of exon 33, which resides in the intracellular domain of the protein. Through cDNA analysis, we determined that this mutation leads to the excision of exon 33 from the mature mRNA transcript, causing a frameshift across the last exon, and a premature stop codon. This is predicted to result in a NOTCH2 protein with loss of 2 of 6 evolutionarily conserved ankyrin repeats and the PEST sequence. The second is a C444Y missense mutation. This causes a loss of a cysteine residue from one of the extra-cellular EGF repeats, which are essential for ligand-receptor binding. Similar mutations in the EGF repeats of JAG1, NOTCH3, DLL3, and FBN1 have all been associated with human disease. This is the first report of disease causing mutations in the NOTCH2 gene, and the first demonstration that Alagille syndrome is a heterogenous disorder.

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Risk haplotype for dyslexia on chromosome 6p22 is associated with a reduced expression of the KIAA0319 gene.
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Dyslexia has an incidence of 5-10%; in school-aged children and is caused in large part by genetic factors. We have recently shown that a haplotype on chromosome 6p22 is significantly associated to dyslexia (Francks et al., 2004). This haplotype spans a 77 kb region of strong inter-marker linkage disequilibrium, encompassing the first four exons of *KIAA0319*, the entire *TTRAP* gene and the first exon of *THEM2*. Mutation screening by DHPLC of all exons and predicted promoters did not detect obvious variants that would disrupt any of the three gene functions. We then hypothesised that the risk haplotype might influence gene transcription regulation. To test this hypothesis we used the MassARRAY (Sequenom) platform to determine quantitatively relative differences in allele-specific transcription in cell lines that were heterozygous for the risk haplotype. We identified three lymphoblastoid and three neuroblastoma cell lines, which carry one copy of the risk haplotype and were heterozygous for at least one marker within the transcript of each of the three genes. Heterozygous markers in proximity of the promoters were also analysed in immuno-precipitated chromatin (haploChIP assay) from the three lymphoblastoid cell lines. Antibodies against phosphorylated RNA polymerase II allowed us to isolate and to use for allele-specific quantitative analysis those chromatin fragments where transcription initiates. The markers analysed for *TTRAP* and *THEM2* showed no quantitative differences between transcripts generated either on the risk or non-risk haplotypes. Conversely, all the markers used for *KIAA0319* showed consistently a transcription reduction of about 40% associated to the risk haplotype. These data show, for the first time, a relation between a genetic background and a biological mechanism that might be involved in the development of dyslexia. We are now working on the identification of the aetiological variant responsible for the reduced expression of the *KIAA0319* gene using different strategies, including gene reporter and EMSA assays.

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A Deletion in DCDC2 on 6p22 is Associated with Reading Disability. *H. Meng[1], S.D. Smith[2], K. Hager[3], M. Held[1], J. Liu[4], R.K. Olson[5], B.F. Pennington[6], J.C. DeFries[5], J. Gelernter[7], T. OReilly-Pol[1], S. Somlo[8], P. Skudlarski[1], S. Shaywitz[1], B. Shaywitz[1], K. Marchione[1], G.P. Page[9], J.R. Gruen[1].* 1) Dept Pediatrics, Yale Univ, New Haven; 2) Munroe Meyer Inst, Univ of Nebraska, Omaha; 3) WM Keck Foundation Biotech Resource Lab, Yale Univ, New Haven; 4) SoftGenetics, State College; 5) Univ of Colorado, Boulder; 6) Univ of Denver, Denver; 7) Dept of Psychiatry, Yale Univ, New Haven; 8) Depy of Internal Medicine, Yale Univ, New Haven; 9) Dept of Biostatistics, Univ of Alabama, Birmingham.

DYX2 on 6p22 is the most replicated reading disability locus. We previously confined DYX2 to a 1.5Mb region surrounding a STR marker, JA04. We now report a peak of single-marker transmission disequilibrium with quantitative traits of reading performance in intron 6 of the DCDC2 (doublecortin domain containing 2) gene (P = 0.0003), identified by saturating this region with 147 SNPs markers. We also characterize short haplotypes with clusters of consecutive markers confined to DCDC2 that show significant transmission disequilibrium with several quantitative assessments of reading performance in the context of preserved IQ. Within human brain, DCDC2 is most strongly expressed in temporal lobe, prefrontal cortex, and posterior cingulate gyrus, regions that, in part, have been implicated in the reading process. We hypothesize that based on amino acid sequence homology to doublecortin domains, DCDC2 could direct subcortical neuron migration; differences in DCDC2 expression could therefore disrupt normal brain circuitry connecting these reading centers. Within the deletion, there is a 168bp purine-rich region (98% AG) containing a compound STR encoding multiple copies of putative PEA3 and NF-ATp binding sites. PEA3 and NF-ATp are transcription factors that are active in brain. Loss of this entire regulatory region, as would happen with the common large deletion we found in dyslexics, would likely affect DCDC2 function. Furthermore, polymorphisms of the compound STR, disrupting the PEA3 and NF-ATp sites, are strongly associated with reading performance (P = 0.00002) and may account for variation in reading ability in subjects without the common deletion, or in compound heterozygotes.

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Mutations in Ionotropic AMPA Receptor 3 (GluR3) in Males with X-linked Mental Retardation. *Y. Wu[1], Y. Jiang[1], L. Zhang[1], M. Splaine[1], R. Huganir[2,4], C. Schwartz[3], D. Valle[1,4], T. Wang[1].* 1) Inst. of Genet. Med., Johns Hopkins Univ., MD; 2) Dept. Neurosci. Johns Hopkins Univ., MD; 3) Greenwood Genet. Center, Clemson Univ. SC; 4) Howard Hughes Med. Inst.

Glutamate receptors are the predominant excitatory neurotransmitter receptors in CNS and are essential for the induction and maintenance of long-term potentiation (LTP) and long-term depression (LTD), two cellular models of learning and memory. Ionotropic AMPA receptors (GluR1-4) mediate basal excitatory synaptic transmission and are a major determinant in the strength of synaptic response. Using an X-chromosome cDNA microarray, we identified a significant reduction of GluR3 transcript in a lymphoblast cell line of an XLMR male, which was confirmed by real-time PCR. By screening of an additional 150 unrelated XLMR males, we identified three missense mutations, R450Q, within the ligand-binding domain; R631S, near the channel-lining core; G833R in the transmembrane domain 4 of GluR3. Amino acid residues at all three positions are conserved in evolution from human to zebra fish. None of these mutations were found in a screening of 540 normal control males. GluR3-G833R protein was reduced to <50% of the normal control in transfected HEK293 cells on Western blot. We rescued the mutant protein to near normal levels when we cultured the cells at 30 oC or inhibited proteosome function by lactacystin, suggesting that G833R results in mis-folding and increased degradation of the mutant protein. Using biotin-based membrane protein-pull down, we showed a significant reduction of GluR3-G833R protein on the plasma membrane (~30% of normal control). Using an AMPA-induced receptor endocytosis assay, we found an 80-90% increase in receptor endocytosis for GluR3-R450Q as reflected by the increased amount of internalized GluR3, suggesting a potential change in the binding property of AMPA to GluR3-R450Q. This finding was further supported by the results of a binding kinetic study using 3H-labeled AMPA and GluR3-R450Q in transfected HEK293 cells. Our results indicate that mutations in GluR3 represent a newly recognized cause of X-linked mental retardation.

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Identification of CUL7 mutations in the 3 M syndrome. *C. Huber[1], D. Dias-Santagata[2], A. Glaser[3], J. O'Sullivan[4], K. Wu[2], X. Xu[2], K. Pearce[3], R. Wang[2], G.C.M. Black[4], P.E. Clayton[4], A. Read[4], M. Le Merrer[1], A. Munnich[1], Z.-Q. Pan[2], R. Winter[3], V. Cormier-Daire[1], and the clinical consortium on 3 M syndrome.* 1) Department of Medical Genetics, Hopital Necker, Paris, France; 2) Department of Oncological Sciences and of Human Genetics, the Mount Sinai School of Medicine, New York, USA; 3) Genes, Development and Disease Theme, Institute of Child Health, London, UK; 4) Department of Clinical Genetics, St Mary's Hospital, Manchester, UK.

First described by Miller, McKusick and Malvaux, the 3-M syndrome (OMIM 273750) is an autosomal recessive condition characterized by severe pre- and post-natal growth retardation, facial dysmorphism, large head circumference, normal intelligence and endocrine function. Skeletal changes include long slender tubular bones and tall vertebral bodies. Studying 29 families with 3-M, we first mapped the disease gene to chromosome 6p21.1. We then considered Cullin 7 (CUL7) as a good candidate based on the cul7 gene targeted mouse model characterized by intrauterine growth retardation (IUGR) at late gestational age, with a placenta of reduced size and early death of respiratory distress. By direct sequencing of the CUL7 gene, we identified 25 mutations including 19 non sense and 6 missense mutations. Northern blot and RT-PCR analyses detected expression of CUL7 in fetal and adult tissues including fetal brain and kidney, placenta, trophoblast, osteoblats, chondrocytes and fibroblasts. CUL7 belongs to the cullin family, which is involved in cell cycle regulation, signal transduction, oxygen regulation and DNA repair. CUL7 assembles an E3 ubiquitin ligase complex containing Skp1, Fbx29/Fbw8, and ROC1 and promotes ubiquitination. Using deletion analysis, we show that CUL7 utilizes its central region to interact with the Skp1-Fbx29 heterodimer. Functional studies indicated that the CUL7 nonsense and missense mutations R1445X and H1464P respectively, render CUL7 deficient in recruiting ROC1. This study provides the first evidence of a cullin gene mutation in a human disease and suggests that impaired ubiquitination may play a role in the pathogenesis of IUGR in humans.

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Interferon Regulatory Factor 6 (Irf6), the mouse ortholog of the gene mutated in Van der Woude Syndrome, is essential for craniofacial, skin, and limb development. *C.R. Ingraham[1,2], R. Richardson[3], B. Yang[1], J. Dixon[3], K.J. Trout[1], M.I. Malik[1], M.J. Dixon[3], B.C. Schutte[1].* 1) Departments of Pediatrics and Obstetrics/Gynecology, University of Iowa, Iowa City, IA; 2) New York University School of Medicine, New York, NY; 3) Faculty of Life Sciences, University of Manchester, Manchester, UK.

Mutations in IRF6 cause Van der Woude Syndrome (VWS) and genetic variation in IRF6 confers risk for non-syndromic cleft lip and palate, the most common form of orofacial clefting. To further study IRF6s role in clefting disorders and to create an experimental model for VWS, two independent mutant alleles for Irf6 were created in mouse: one by gene targeting and one by gene trapping. In both strains, Irf6+/- embryos appear normal, but Irf6-/- embryos die within a few hours of birth. Observations were thus made before embryonic day 17.5 (E17.5). E17.5 Irf6-/- mutants had taut skin lacking normal ridges or wrinkles, smaller heads, absent ears, shortened snouts, shortened forelimbs lacking visible digits, and the hindlimbs and tail were fused into one caudal projection. Fused bones were present in all feet. The skin showed abnormal differentiation, with a normal basalis layer, expanded spinosum, and absent granulosum and corneum layers. Irf6 is expressed exclusively in the spinosum layer of wild-type mice and is absent in mutants. We conclude that Irf6 is essential for craniofacial, skin, and limb development in mouse. Further phenotypic analysis revealed cleft palate in all Irf6-/- mice (n=61). Given the abnormal differentiation of skin epithelium and the high expression of Irf6 in the medial edge epithelium of the palate shelves, we hypothesized that palate shelves excised from mutant mice would fail to fuse in vitro. Surprisingly, similar rates of fusion were observed between mutants (n=3) and wild-types (n=5). Thus, the cleft palate in Irf6-/- mice does not appear to be due to a defect in growth or fusion of the palate shelves. Rather, there is a problem with elevation of the palate shelves, and further studies are needed to determine if this is a primary defect, or secondary to the skin defect causing crowding of craniofacial structures.

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The common Bardet-Biedl syndrome type 1 (BBS1) M390R mutation is a hypomorphic allele that protects against embryonic lethality and cardiovascular defects. *R.E. Davis[1], H. Yen[1], M. Tayeh[1], R.F. Mullins[1], M.A. Fath[1,2], C.C. Searby[1,2], D.Y. Nishimura[1,2], B. Yang[1], D. Slusarski[1], V.C. Sheffield[1,2].* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute.

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder resulting in obesity, retinopathy, polydactyly, cognitive impairment, congenital heart defects, and renal abnormalities. Patients with this disorder display variable expressivity. To date, eight BBS genes have been reported. *BBS1* is the most commonly mutated gene and a single mutation (M390R) is involved in ~80% of cases. Our laboratory developed mouse models with a *Bbs1* null allele, as well as a knock-in model with the *Bbs1* M390R allele. Homozygosity for the null allele resulted in embryonic lethality, while homozygous knock-in mice were produced at the expected Mendelian ratio. M390R *Bbs1* mice recapitulate primary aspects of the human phenotype including obesity, photoreceptor cell death, and male infertility. *Bbs1* knock-in mice also have diminished olfaction and leptin resistance. These data indicate that the M390R allele has partial functional activity, as mice with this allele are rescued from the embryonic lethality observed in *Bbs1* null mice.

To further evaluate developmental aspects of *BBS1* deficiency, we analyzed the phenotype resulting from antisense morpholino (MO) knockdown of zebrafish *bbs1* expression. Zebrafish *bbs1* knockdown results in cilia and organelle transport defects, as well as cardiac abnormalities resulting in enlarged ventricles and atria, and pericardial edema. The morphants do not survive past 5 days post-fertilization. Normal *bbs1* RNA rescues all aspects of the MO knockdown phenotype. However, RNA containing the M390R mutation rescues the cardiac phenotype but not the ciliated organ defect or the organelle transport defect. Our combined data demonstrate that the *BBS1* M390R missense mutation is a hypomorphic allele that protects against embryonic death and defects in cardiac development compared to *BBS1* null alleles.

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Identification of the first gene causing Meckel syndrome, MKS 1. *M.M. Kyttälä[1,2,3], J.E. Tallila[1], R. Salonen[4], M. Kestilä[1], L. Peltonen[1,2,3].* 1) Department of Molecular Medicine, Biomedicum, NPHI, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Department of Human Genetics, University of California Los Angeles, USA; 4) Department of Medical Genetics, Väestöliittö, Finland.

Meckel syndrome (MKS) is an autosomal recessive developmental disorder, diagnosed in most populations. It is characterized by three primary defects: cystic and fibrotic changes of liver and kidney, occipital meningo encephalocele and polydactyly. MKS affects 1:9000 births in the Finnish population and was mapped in Finnish families to chr17q23 (MKS1, MIM 249000). Two other loci for MKS have been identified on chr11q13 (MKS2, MIM 603194) and on 8q24 (MKS3, MIM 607361) in families representing different ethnic backgrounds. We report here the identification of the first MKS gene, MKS1, representing a previously unknown transcript of a 14 kb gene on 17q. The coding region shows across species homology and the structure of mouse gene is identical to that of human gene with 18 exons. The Finnish MKS1 major mutation causes a defective splicing and leads to a transcript missing one exon and the frameshift after the exon deletion. This altered splicing can be seen also in mRNA extracted from different tissues of Finnish MKS patients. All Finnish patients are homozygous for the mutation and we found this same mutation in a homozygous form in three non-Finnish patients from different Caucasian populations. We have also identified two additional mutations in this gene: one splice site mutation and one five bp insertion in exon 1 in a German MKS family. In situ hybridization established low level ubiquitous expression of the Mks1-gene in mouse embryonic tissues. Higher expression was observed in limb buds, brain, kidney and liver: organs showing characteristic malformations for MKS. The expression array analyses accompanied by bioinformatics studies should provide clues of the metabolic pathways associated with the novel MKS gene and disturbed in this lethal malformation syndrome. Further characterization of the MKS gene should reveal a pathway or signalling cascade, crucial for the normal embryonic development.

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Mutations in *UBR1*, encoding an E3 ubiquitin ligase of the N-end rule pathway, cause Johanson-Blizzard syndrome. *M. Zenker[1], J. Mayerle[2], A. Tagariello[1], K. Zerres[3], P.R. Durie[4], G. Huelskamp[5], C. Guzman[6], H. Rehder[7], F.A. Beemer[7], B. Hamel[7], P. Vanlieferinghen[7], R. Gershoni-Baruch[7], M.W. Vieira[7], Y.T. Kwon[8], A. Varshavsky[9], M.M. Lerch[2], A. Reis[1].* 1) Inst of Human Genetics, Univ Erlangen, Germany; 2) Dept of Gastroenterology, Univ Greifswald, Germany; 3) Inst of Human Genetics, Univ Aachen, Germany; 4) Dept of Pediatrics, Univ Toronto, Canada; 5) Dept of Pediatrics, Univ Muenster, Germany; 6) National Children's Hospital, Univ Costa Rica, San Jose, Costa Rica; 7) Depts of Genetics, Univ Vienna, Austria; Univ Utrecht and Nijmegen, The Netherlands; CHU Clermont-Ferrand, France; Rambam Medical Center, Haifa, Israel; and Univ Sao Paolo, Brasil; 8) Center of Pharmacogenetics, School of Pharmacy, Univ Pittsburgh, USA; 9) Div of Biology, California Inst of Technology, Pasadena, USA.

Johanson-Blizzard syndrome (JBS; OMIM 243800), an autosomal recessive multisystem disorder, is characterized by the combination of congenital exocrine pancreatic insufficiency and aplasia of the alae nasi, as well as multiple facultative features, including scalp defects, imperforate anus, deafness, hypothyroidism, dental anomalies, genitourinary malformations, and mental retardation. To determine the molecular basis of JBS, we mapped this disease to 15q14-21.1, using a genome-wide linkage scan. We identified truncating mutations in the gene *UBR1* in 12 unrelated JBS families. *UBR1* encodes one of several E3 ubiquitin ligases of the N-end rule pathway, a ubiquitin-dependent proteolytic pathway whose substrates include proteins with destabilizing N-terminal residues. This pathway is present in both prokaryotes and eukaryotes. Its known functions include the regulation of peptide import, apoptosis, chromosome segregation, meiosis, cardiovascular development, and leaf senescence in plants. We show that *ubr1*(-/-) mice, whose previously reported phenotypes include reduced weight and behavioural abnormalities, have an exocrine pancreatic insufficiency with impaired stimulus-secretion coupling and increased susceptibility to pancreatic injury. Our results indicate that the perturbation of UBR1-dependent ubiquitylation is the primary cause of JBS.

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Trps1 is a repressor of the gene for the cartilage matrix component Perlecan, *Hspg2*. *H.-J. Luedecke, P. Brega, F. Kaiser, B. Horsthemke.* Institut für Humangenetik, Universitätsklinikum, Essen, Germany.

Heterozygous mutations of the *TRPS1* gene cause the tricho-rhino-phalangeal syndrome (TRPS), which is characterized by craniofacial and skeletal abnormalities. Mice carrying a targeted deletion of the GATA zinc finger encoding exon (*Trps1*$^{+/-}$) develop mild signs of this disease, whereas homozygous mutant mice (*Trps1*$^{-/-}$) die of neonatal respiratory failure resulting from spine and rib abnormalities. To identify target genes of the TRPS1 transcription repressor, we compared the expression profiles of limbs from three wild-type and two *Trps1*$^{+/-}$ E11.5 embryos from the same litter using Affymetrix MOE430A oligonucleotide arrays. The gene for the core protein of the heparan sulfate proteoglycan Perlecan, *Hspg2*, was among the genes found to be differentially expressed. In agreement with Perlecan's previously published developmental expression pattern, the *Hspg2* transcript was absent in E11.5 wild-type embryos but present in significant amounts in heterozygotes. This result was confirmed by quantitative real-time PCR analysis of limbs from two wild-type and eight *Trps1*$^{+/-}$ embryos from three additional litters. To examine whether *HSPG2* is a direct target of TRPS1, we used 1,711 bp from the upstream promoter region of the human *HSPG2* gene, which contains nine GATA1 motifs, to control the expression of an SV40-promoter driven luciferase reporter gene. We observed a TRPS1 dosage-dependent repression of the luciferase activity in HeLa cells co-transfected with the reporter and a TRPS1 encoding plasmid. Perlecan is a component of the extracellular matrix of many organs and tissues. Increased as well as decreased deposition of Perlecan is associated with defects in several tissues such as the nasal septum, oral epithelia, the growth plates of the developing bone, and the articular surface of the bone, which are affected in TRPS. Our results suggest that up-regulation of *HSPG2* expression may explain some of the clinical signs of TRPS like bulbous tip of the nose, cleft palate, cone-shaped epiphyses or early-onset osteoarthritis.

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Patterns of BDNF/NTRK2 variants and BDNF protein blood levels in the susceptibility to eating disorders. *X. Estivill[1,2,3], M. Ribasés[1], J.M. Mercader[1], H.C. Howard[1], B. Puchau[1], F. Fernández-Aranda[4], M. Bayés[1,2], M. Gratacòs[1,2].* 1) Genes and Disease Program, Center for Genomic Regulation (CRG), Barcelona, CATALUNYA, Spain; 2) CeGen - Spanish Genotyping Network, Barcelona, CATALUNYA, Spain; 3) Pompeu Fabra University (UPF) Barcelona, CATALUNYA, Spain; 4) Psychiatric Service, Ciutat Sanitaria Bellvitge, LHospitalet, CATALUNYA, Spain.

Eating disorders (ED), such as anorexia nervosa (AN) and bulimia nervosa (BN), predominantly affect women and adolescents, and have a high prevalence and mortality rate in European populations. Although environmental factors play an essential role in the aetiology of ED, there are strong evidences of a genetic participation in the predisposition and development of AN and BN. Experiments on murine models and pharmacological studies in rats indicate the potential role of BDNF in the regulation of food intake and body weight as an anorexigenic factor. We have performed several case-control studies finding a consistent association between the -270C/T BDNF SNP and bulimia nervosa (BN), and the Val66Met variant to both AN and BN in different European populations. Furthermore, haplotype relative risk analysis and the transmission disequilibrium test (TDT) in ED trios for BDNF alleles have been confirmed in a large sample of trios from different European countries. We have analyzed SNPs with a minor allele frequency higher than 0.10, covering the entire BDNF gene using the SNPlex technology in 174 ED patients and 174 sex-matched unrelated controls, permitting a dense and targeted genetic characterization of the variability of BDNF in ED. We have also screened the entire NTRK2 gene in patients with ED and found evidence of association of a specific NTRK2 haplotype with binge-eating/purging AN, and a reduced frequency of another haplotype in BN patients. Finally, we have assessed BDNF plasma levels in 50 discordant sib pairs with ED and found that BDNF levels were significantly higher in ED patients than in their unaffected sibs, irrespectively of the Val66Met genotype. The data strongly indicate that BDNF participates in the genetic susceptibility to ED and strongly argue for a role of BDNF signaling in eating behavior and body weight regulation.

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Disentangling depression and apolipoprotein E. *M.A. Slifer[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Apolipoprotein E isoforms play an important role in many neuropsychiatric disorders including Alzheimer disease, Parkinson disease, cerebrovascular ischemic disease, and traumatic brain injury recovery. Geriatric depression is a highly prevalent neuropsychiatric mood disorder thought to be related to neurologic injury in the elderly. Geriatric depression is clinically paramount as an independent risk factor, across co-morbidities, for poor outcomes including mortality. Importantly geriatric depression is under-recognized, but when recognized, it is amenable to treatment. Several reports have linked Apolipoprotein E isoforms (ApoE) with geriatric depression (Caselli 2004, Flicker 2004, Laveretsky, Muller-Thomsen 2002, Nebes 2001, Stewart 2001). Others reports failed to identify an association (Liu 2002, Mauricio 2000, Harwood 1999, Papassotiropoulos 1999, Levy 1999, Forsell 1997, Lyketsos 1997). In this study, we present results from the largest study to date of ApoE and depression. Unlike previous studies, we assess mood symptoms, cognitive symptoms and ApoE genotype for our analyses. In the overall Collaborative Alzheimer Disease case-control data set (n=999), ApoE-4 is associated with depression as reported in 30.7% of ApoE-4 carriers versus 20.7% of ApoE-4 non-carriers ($p<0.01$). However, the presence of dementia completely explains the observed effect. When stratifying on dementia, there is no significant difference in depression by ApoE genotype in either the Alzheimer cases (n=491) or controls (n=508). This suggests that the inconsistent results among prior studies may be due to unrecognized confounding. Furthermore, this study demonstrates the importance of accounting for co-morbid dementia in the assessment of mood symptoms in the elderly.

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A Scan of Chromosome 10 Identifies Novel Candidate Genes Showing Strong Association to Late-Onset Alzheimer's Disease. *J.S.K. Kauwe III[1], A. Grupe[2], Y. Li[2], C. Rowland[2], P. Nowotny[1], A.L. Hinrichs[1], S. Smemo[1], L. Doil[2], K. Tacey[2], P. Holmans[3], J. Hardy[5], M. O'Donovan[3], S. Lovestone[6], L. Jones[3], J. Morris[1], L. Thal[4], M. Owen[3], J. Williams[3], A. Goate[1].* 1) Washington Univ Sch of Med, St Louis, MO; 2) Celera Diagnostics, Alameda, CA; 3) Cardiff Univ, Wales College of Med, Cardiff, UK; 4) Univ of California, San Diego, La Jolla, CA; 5) National Institute on Aging, Bethesda, MD; 6) King's College London, London, UK.

Evidence of linkage to late-onset Alzheimers disease (LOAD) has been observed on chromosome 10, implicating a wide region and at least one disease susceptibility locus. Although significant associations with several biological candidate genes on chromosome 10 have been reported, these findings have not been consistently replicated and remain controversial. We performed a chromosome 10 specific association study with 1,430 single nucleotide polymorphisms (SNPs, allele frequency 2%). The scan covered 728 genes with one or more markers per gene. Initial tests were performed in a Caucasian case-control sample from the St. Louis area with 419 LOAD cases and 377 aged-matched controls. Markers that showed significant association in the exploratory analysis were followed up in two other Caucasian case-control sample sets to confirm the initial association. The replication case-control sets included a total of 670 LOAD cases and 819 controls, collected in the San Diego area and in the UK. Of the 1,398 markers tested in the exploratory sample, 69 reached significance (P=<0.05). Of these, 5 markers were replicated at P<0.05. One marker, rs498055, located in a gene homologous to RPS3A, was significantly associated with AD in each of the three case-control series, with an allelic P-value of 0.00004 (OR=1.3) in the meta-analysis. This marker was also tested in a case-control sample derived from our linkage sample (P=0.0165 OR=1.257). These results indicate that variants in the RPS3A homolog and haplotypes in the RPS3A homolog region are associated with LOAD and implicate this gene, adjacent genes or other functional variants (e.g. non-coding RNAs) in the pathogenesis of this disorder.

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Polymorphisms in paraoxonase are associated with Alzheimer Disease in African Americans and Caucasians.

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Epidemiological and autopsy studies suggest an involvement of cerebrovascular atherosclerosis in the etiology of Alzheimer disease (AD). The serum enzyme paraoxonase, encoded by 3 PON genes on chromosome 7q21 has been implicated in several vascular disorders and recently in AD. We conducted a high density SNP association study to investigate the association between the PON gene cluster and AD in African American and Caucasian subgroups of the MIRAGE Study. The sample included 243 African American and 277 Caucasian AD cases, their non-demented sibling controls (N=143, N=329), and unrelated spouse/neighborhood controls (N=81, N=124). We used family-based tests (FBAT) in the sib pairs and a generalized estimating equation approach in the full data set to test for association. Of the 29 PON SNPs genotyped, 7 SNPs were associated with AD (0.0002p0.03) in the full African American sample and 2 SNPs were associated with AD in the Caucasian sibships 0.01p0.04). In the combined family sample, single-SNP FBAT analysis revealed significant evidence of association for 4 SNPs (0.007p0.03), and haplotype FBAT analysis using sliding windows of 2-5 SNPs identified AD associated haplotypes throughout the PON gene cluster (0.00009p0.04). The strongest association was found in the region spanning the PON1 proximal promoter, where a common haplotype (20% frequency) was transmitted disproportionately to AD probands. These data suggest that a genetic variant in the PON1 promoter influences AD risk in both Caucasians and African Americans. Association was observed also in transcribed regions of PON1, PON2 and PON3. The multiplicity of sites within the PON locus associated with AD may be due to extensive LD across the region or the existence of multiple functional variants affecting AD risk. These findings are likely to be robust and replicable because the observations were made in two independent and ethnically diverse populations. Given the well established role of paraoxonase in atherosclerosis, these results suggest that atherosclerosis may be a part of the etiology of AD.

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Dose effect of Apolipoprotein A-II (TG) repeat on age-at-onset in APOE-4(+) late-onset Alzheimer disease by splicing modification. *K. Kamino[1], T. Kida[1], T. Tanaka[1], T. Kudo[1], M. Yamamoto[1], R. Kimura[1], H. Akatsu[2], T. Uema[3], H. Yoneda[4], T. Miki[5], H. Hattori[6], M. Imagawa[7], Y. Ihara[8], S. Ohta[9], M. Takeda[1].* 1) Post-Genomics & Diseases, Osaka Univ Grad Sch Med, Suita, Japan; 2) Choju Medical Institute, Fukushimura Hosp, Toyohashi, Japan; 3) Psychiatry, Osaka General Medical Center, Osaka, Japan; 4) Neuropsychiatry, Osaka Medical College, Takatsuki, Japan; 5) Geriatric Medicine, Ehime Univ Sch Med, Toon, Japan; 6) Psychiatry, National Hosp for Geriat Med, Ohbu, Japan; 7) Imagawa Clinic, Osaka, Japan; 8) Neruopathology, Fac Med, Univ Tokyo, Tokyo, Japan; 9) Biochemistry and Cell Biology, Institute of Gerontology, Nippon Medical School, Kawasaki, Japan.

It has been noted that patients with late-onset Alzheimer disease (LOAD) show decreased plasma apolipoprotein A-II (apoA-II) levels, but it remains undetermined whether this decrease reflects a preventive effect of apoA-II or the resultant outcome of nutrition and the physical condition. The apolipoprotein A-II (APOA2) gene showed 9 major haplotypes, which can be grouped into 4 haplotype groups in Japanese subjects. Haplotype-based alleles were in Hardy-Weinberg equilibrium in both 564 sporadic LOAD and 746 age-matched control subjects. No haplotype-based alleles were associated with LOAD, and age-at-onset, in normal distribution, tended to correlate with the dose of the apolipoprotein E (APOE)-4 allele in all LOAD subjects. Nonetheless, in 234 patients with APOE-4(+) LOAD, the age-at-onset significantly showed an inverse correlation with the (TG) dose of intron 2 microsatellite of the APOA2 gene ($p<0.005$), and haplotype-basd allele dose did not contradict to the effect of the (TG) dose. In vitro transfection in COS-7 cells of minigene vectors harboring exon 3 and its nearby sequence of the APOA2 gene revealed that the (TG) number was correlated with the amount of the transcript lacking exon 3, and the same effect was also found by genomic APOA2 expression vectors. These results indicate that the APOA2 gene modifies age-at-onset in APOE-4(+) LOAD, explained by altered splicing of the APOA2 exon 3, and suggest that impaired splicing efficiency is involved in the occurrence of LOAD.

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Metabolic stress induces cell death in Parkinson disease cybrids and fibroblasts. *J. van der Walt[1], M. Noureddine[1], P. Trimmer[2], J. Bennett[2], J. Stajich[1], B. Scott[1], M. Stacey[1], W.K. Scott[1], E.R. Martin[1], Y. Li[1], M. Hauser[1], M.A. Pericak-Vance[1], J.M. Vance[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Center for the Study of Neurodegenerative Diseases and Department of Neurology, University of Virginia, School of Medicine, Charlottesville, VA.

Substantial evidence suggests that mitochondrial (mt) impairment may be involved in Parkinson disease (PD) pathogenesis. Complex I is the major site of reactive oxygen species (ROS) generation within the mitochondrion during metabolism leading to damage of DNA, lipids and proteins. Biochemical analyses using cybrids have demonstrated impairment of complex I in PD cases indicating that mtDNA may cause the deficit in energy production. For the purpose of this study, we created two cell models to examine mt function. Cybrids were created by fusing donor platelets with SH-SY5Y cells and fibroblast cultures were created from skin biopsies. In order to test mt function in cells derived from PD patients and controls, we induced metabolic stress in cell culture and observed cell growth. Galactose media was used to force cells into utilizing mt oxidative phosphorylation (OXPHOS) to generate ATP rather than glycolysis. We observed six out of nine PD cybrid lines and 2 out of five age-matched control cybrid lines that were unable to sustain growth in the galactose media after 48-96 hours. Western blot analysis of OXPHOS complexes demonstrated that COX2 and ND6 were either absent or found at low levels in cybrids lines that died under galactose culture. All other cybrids that survived in galactose showed normal levels of mt protein. We also incorporated a mtDNA resequencing kit (MitoChip) to detect mutations within one PD cybrid. Through this method, we were able to detect one heteroplasmic mutation within the ND1 gene. Similarly, cell growth of PD fibroblast lines (14/14) under galactose were arrested after 9 days while all control lines (4/4) proliferated. These preliminary results suggest that mt impairment affects cell growth in cell culture models of PD. Further investigation is needed to elucidate whether cell death is caused by decreased ATP production, increased oxidative stress or a combination of both mechanisms.

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High-resolution Whole-genome Association Study of Parkinson's Disease. *D.M. Maraganore[1], M. de Andrade[2], T.G. Lesnick[2], K.J. Strain[2], M.J. Farrer[3], W.A. Rocca[1,2], K. Pant[4], K.A. Frazer[4], D.R. Cox[4], D. Ballinger[4].* 1) Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN; 2) Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; 3) Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; 4) Perlegen Sciences, Inc., Mountain View, CA.

We performed a two-tiered, whole-genome association study of Parkinsons disease (PD [MIM 168600]). For tier 1, we individually genotyped 248,535 uniformly spaced single nucleotide polymorphisms (SNPs) in 443 sibling pairs discordant for PD. For tier 2a, we individually genotyped 1,518 PD-associated SNPs ($p < 0.01$ in tier 1) and 311 genomic control SNPs in 332 matched case-unrelated control pairs. We identified 25 SNPs that were associated with PD ($p < 0.01$) in both tier 1 and tier 2 samples. For these 25 SNPs, we combined data from the case-unaffected sibling pair (tier 1) and case-unrelated control pair (tier 2) samples and employed a liberalization of the sibling transmission disequilibrium test to calculate odds ratios, 95% confidence intervals, and p-values. Eleven of the 25 SNPs were associated with PD in the combined data and had the same direction of the effect in tier 1 and tier 2a. A SNP within a semaphorin-related gene had the lowest combined p-value ($p = 7.62 \times 10\text{-}6$). Proteins encoded by this class of genes play important roles in neurogenesis and neuronal apoptosis, consistent with existing hypotheses regarding PD pathogenesis. A second SNP tagged the PARK11 late onset PD susceptibility locus ($p = 1.70 \times 10\text{-}5$). We also selected for genotyping in tier 2b additional SNPs that in tier 1 were borderline significant ($p < 0.05$) but which tested a priori biological and genetic hypotheses regarding susceptibility to PD (n = 1,312 SNPs). In analysis of the combined tier 1 and tier 2b data, one of these SNPs tagged the PARK10 late onset PD susceptibility locus ($p = 9.07 \times 10\text{-}6$). Independent replication across populations will clarify the role of the genomic loci tagged by these SNPs in conferring PD susceptibility.

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A frameshift mutation in Disrupted in Schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder. *N.A. Sachs[1], S.E. Holmes[1], P. Sklar[2], S. Waggoner[2], M.T. Pato[3], C.N. Pato[3], D.B. Wildenauer[4], S.G. Schwab[4], F. Dickerson[5], R.H. Yolken[1], F.J. Leister[1], V.L. Nimgaonkar[6], A. Sawa[1], C.A. Ross[1], L.E. DeLisi[7], R.L. Margolis[1].* 1) Johns Hopkins University Sch of Med, Baltimore; 2) Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; 3) Department of Psychiatry, State University of New York, Syracuse, NY; 4) School of Psychiatry and Clinical Neurosciences, University of Western Australia, Perth, Australia; 5) Stanley Research Center, Sheppard Pratt Health System, Baltimore; 6) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 7) Department of Psychiatry, New York University, NY, NY.

In a large Scottish pedigree, a balanced translocation t(1:11)(q42.1q14.3) segregates with major mental illness, including schizophrenia, bipolar disorder, and recurrent major depression. The translocation is predicted to lead to loss of the C-terminal region of the protein product of Disrupted In Schizophrenia 1 (DISC1), a gene located on 1q42.1. DISC1 has been functionally implicated in several processes, including neurodevelopment. We sequenced portions of DISC1 in 28 unrelated probands with schizophrenia and six unrelated probands with schizoaffective disorder. We detected a 4 bp deletion at the extreme 3 end of exon 12 in a proband with schizophrenia. The mutation has been detected in a sib with schizophrenia, a sib with schizoaffective disorder, and the father with possible major depression, while the mutation has not been detected in 2035 cases or 2182 controls. The mutant transcript is detectable, but at a reduced level, in lymphoblastoid cell lines from three of four mutation carriers. The mutation is predicted to cause a frameshift and encode a truncated protein with nine abnormal C-terminal amino acids. While previous research demonstrates that DISC1 interacts with NUDEL, transient transfection and immunoprecipitation demonstrated that the mutated form of DISC1 does not interact with NUDEL. These findings are consistent with the possibility that mutations in the DISC1 gene may increase the risk for schizophrenia and related disorders.

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Increased expression in dorsolateral prefrontal cortex of CAPON in schizophrenia and bipolar disorder. *L.M. Brzustowicz[1,4], B. Xu[1], N. Wratten[1], E.I. Charych[2], S. Buyske[1,3], B.L. Firestein[2].* 1) Departments of Genetics; 2) Cell Biology and Neuroscience, and; 3) Statistics, Rutgers University, Piscataway, NJ; 4) Department of Psychiatry, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ.

We have previously reported linkage of markers from chromosome 1q22 to schizophrenia, a finding supported by several independent studies. Within this linkage region, we have identified significant linkage disequilibrium (LD) between schizophrenia and markers within the gene for carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON). Prior sequencing of the 10 exons of CAPON failed to reveal a coding mutation associated with illness. We have screened a human fetal brain cDNA library and identified a new isoform of CAPON, which consists of only the terminal two exons of the gene, and have verified the expression of the predicted protein in human dorsolateral prefrontal cortex. We have examined the expression levels of both the 10-exon CAPON transcript and the new isoform in post-mortem brain samples from the Stanley Array Collection. Quantitative real-time PCR analysis of RNA from the dorsolateral prefrontal cortex in 105 subjects, 35 each with schizophrenia, bipolar disorder, and psychiatrically normal controls, revealed significantly ($p<0.005$) increased expression of the new isoform in both schizophrenia and bipolar disorder. Furthermore, this increased expression was significantly associated ($p<0.05$) with genotype at the three SNPs previously identified as being in LD with schizophrenia. Based on the known interactions between CAPON, neuronal nitric oxide synthase (nNOS), and proteins associated with the N-methyl-D-aspartate receptor (NMDAR) complex, over-expression of either CAPON isoform would be expected to disrupt the association between nNOS and the NMDAR, leading to changes consistent with the NMDAR hypofunctioning hypothesis of schizophrenia. This study adds support to the role of CAPON in schizophrenia, produces new evidence implicating this gene in the etiology of bipolar disorder, and provides insight into the potential mechanism of action of CAPON in psychiatric illness.

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Association of *GABRA4* and *GABRB1* in African American autism families. *A.L. Collins[1], D.Q. Ma[1], P.L. Whitehead[1], E.R. Martin[1], H.H. Wright[2], R.K. Abramson[2], J.P. Hussman[3], J.R. Gilbert[1], M.L. Cuccaro[1], M.A. Pericak-Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) The Hussman Foundation, Ellicott City, MD.

Autism is a neurodevelopmental disorder characterized by impairment in social interaction and communication as well as repetitive behavior. It is thought to be a complex genetic disorder, caused by many interacting genes. While many candidates exist, to date no single gene has consistently been identified as a major player in the disorder. Multiple lines of data including alterations in levels of GABA and the GABA receptors in autistic patients indicate that the GABAergic system, which is responsible for synaptic inhibition in the adult brain, may be involved in autism. Previous studies in our lab have indicated association of non-coding single nucleotide polymorphisms (SNPs) with a GABA receptor subunit gene on chromosome 4, *GABRA4*, as well as interaction between SNPs in *GABRA4* and *GABRB1* (also on chromosome 4), within caucasian autism patients. Studies of genetic variation in African American (AA) autism families are rare despite data supporting similar involvement of this racial group. We screened SNPs in the *GABRA4* (N=7) and *GABRB1* (N=5) in our AA dataset of 48 families and have found rs2280073 and rs13151759 (r^2=0.51;*GABRA4*) and hcv2119841 ($r^2$0.016;*GABRB1*) all show significant allelic association with autism in the AA families, p=0.0035, 0.034 and 0.041, respectively. Interestingly, rs13151759 is located in the region homologous to the minimal promoter region in mouse, suggesting a potential functional change. These results confirm our earlier findings implicating *GABRA4* and *GABRB1* as genes contributing to autism susceptibility and extend autism genetic studies beyond a single racial-ethnic group.

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Using haplotype maps to improve power of whole genome association studies with fixed sets of markers. *I. Pe'er[1,2], P.I.W. deBakker[1,2], J. Maller[1,2], K. Jones[3], D.M. Altshuler[1,2], M.J. Daly[1,2].* 1) Broad Institute, MIT & Harvard, Cambridge, MA; 2) Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA; 3) Affymetrix Inc., Santa Clara, CA.

Whole genome association studies are an increasingly practical strategy for dissecting the heritable causes of common complex disease. With the goal of scanning the lions share of the 107 polymorphic sites in the Human genome for association, challenges involve both practical throughput as well as statostocs. Practically, the economically-feasible scale of SNP genotyping allows directly typing only a small fraction of these sites, with technology and economy imposing this fraction to be a fixed set of markers in a whole-genome genotyping product. Statistically, the true association signal is hidden by many random ones. Recent data resources of genomewide variation patterns allows tackling these obstacles, in order to empower association studies performed using a fixed set of markers. We developed theoretical framework, software implementation, and data summaries to thereby improve association testing in several respects: (i) We list linkage-disequilibrium-based correlations between typed and untyped SNP alleles. These imply a small fraction of SNPs actually captures a much larger set of variants; (ii) We list and apply predictors for each untyped allele based on several typed ones, This increases the extent of variation tested for association with the same set of typed SNPs; and (iii) We weigh different association tests according to a Bayesian prior. We develop a neutral such prior, uniform over all SNPs, that dictates promotion of tests that examine more correlated alleles, i.e of variants that are in denser bins of linkage disequilibrium. We examine these approaches and their contribution to improved statistical power when applied to the Affymetrix 100k and 500k mapping arrays.

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Coverage and Power in Genome-wide Association Studies. *E. Jorgenson, J. Witte.* Epidemiology & Biostatistics, University of California, San Francisco, San Francisco, CA.

Large sets of SNPs are becoming available for genome-wide association studies. The extent to which these SNP sets provide information about unobserved SNPs (coverage) is currently unknown. We can use information from resequencing efforts focused on specific regions of the genome and groups of genes as a gold standard for determining the coverage that large genome-wide SNP sets provide. Since less than complete linkage disequilibrium between the causal locus and observed SNPs will result in a reduction in power, quantifying sequence coverage will provide a metric for the reduction in power to be expected when using a particular SNP set to perform genome-wide association studies. We compare average and cumulative distribution measures of sequence coverage, using real SNP data, to determine the reduction in power. Measures based on the cumulative distribution reveal that power is lower than expected under commonly used average metrics. This has important implications for genome-wide association studies, as average metrics, including the average maximum r^2, can inflate power estimates and lead to a higher than expected false negative rate.

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Statistical power provided by random SNP subsets in whole genome case-control association studies. *R. Lazarus[1], W. Qiu[1], B.A. Raby[1], E.K. Silverman[1], P. Kraft[2], C. Rakovski[2], N. Laird[2], S.T. Weiss[1].* 1) Channing Laboratory, Boston, MA; 2) Harvard School of Public Health, Boston, MA.

Whole genome association experiments in which hundreds of thousands of random SNPs are genotyped using extremely high-throughput technologies are now feasible and attractive for complex disease research. Where a large sample has been completely sequenced, adequate power can be obtained using small, carefully chosen sets of tag SNPs, but the statistical power for genome wide case-control association studies using randomly chosen subsets of SNPs is not well understood. We simulated a very large number of case-control studies under alternative hypotheses to obtain empirical estimates of statistical power. Samples were drawn from 14400 diplotypes implied by random mating of 120 CEPH parental HapMap chromosomes, to ensure that realistic LD patterns and haplotype distributions were preserved. Only SNPs with minor allele frequency (MAF) >= 0.05 (n=7461) were considered. Over a range of alternative hypothesis odds ratios (OR), samples of cases and controls were drawn for every possible disease susceptibility locus (DSL). The smallest p-value from testing every genotyped SNP over a range of random SNP subset sizes was recorded, and compared to a Bonferroni adjusted threshold (0.05/n where n=number of tests) to control familywise error. The entire procedure was repeated over each of the 10 ENCODE regions. Power estimates were averaged over repeated simulations at each combination of factors to improve precision, because we noted that random subsets, particularly at low densities, provide highly variable power. For DSL with MAF > 0.2, on average, in 500 cases and 500 controls, 80% statistical power is obtained from a random SNP subset of 1.5% for OR=2.0, and 3% for OR=1.75. Assuming about 5 million SNPs >= 0.05 MAF in the human genome, a 3% (n=150,000) sample of randomly selected SNPs can be expected to provide adequate power for DSL SNPs > 0.2 MAF with true OR=1.75 or greater. The situation for SNPs below 0.2 MAF is more problematic. For DSL SNPs between 0.05 and 0.1 MAF, a 10% random SNP density is required to provide 80% power to detect a true OR=3.0.

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Forget replication: Joint analysis is more efficient for whole genome association studies. *A.D. Skol, L.J. Scott, G.R. Abecasis, M. Boehnke.* Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Whole genome association is a promising approach to identify common genetic variants that predispose to human diseases. Because of the high cost of genotyping hundreds of thousands of markers on thousands of subjects, whole genome association studies will often follow a staged design in which a proportion of the available samples are genotyped on a large number of markers in stage 1, and a small proportion of these markers are followed up by genotyping them in the remaining samples in stage 2. The standard strategy for analyzing such two-stage data is to view stage 2 as a replication study, and to focus on findings that reach statistical significance when stage 2 data are considered alone. An alternative strategy for two-stage data is to analyze all available data jointly. We compare the power of replication-based and joint analysis for two-stage designs. We find that regardless of the two-stage design used, and despite the need to use more stringent significance levels, analyzing the data from both stages jointly is always more powerful than treating stage 2 as a replication study for stage 1, and that in many cases joint analysis results in substantially greater power. Consistent with previous work by Satagopan and colleagues (2002, 2004), we further demonstrate that joint analysis of two-stage data can attain nearly identical power to that of the one-stage design in which all markers are genotyped on all samples, while requiring only a fraction of the genotyping effort. For example, a one-stage design which genotypes 1000 cases and 1000 controls on 300k markers (600 million genotypes) has 75% power to find a disease predisposing variant with a multiplicative genotype relative risk of 1.4, prevalence .10, and risk allele frequency .50 in the controls. Nearly identical power (74%) can be achieved by joint analysis when genotyping 40% of the sample in stage 1 and following up 5% of the markers in stage 2. Analyzing these same two-stage data using a replication-based analysis achieves only 51% power. We suggest that for two-stage whole genome association studies, we should forget replication, and use joint analysis as the standard analysis strategy.

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Logistic regression protects against population structure in genetic association studies. *D. Balding[1], E. Setakis[1], H. Stirnadel[2].* 1) Dept Epidemiology/Public Hlth, Imperial Col Sch Medicine, London, United Kingdom; 2) Worldwide Epidemiology, GlaxoSmithKline, Harlow, United Kingdom.

Although there has been much interest in the effects of population structure on genetic association studies, and in the relative merits of different prophylactic statistical methods, there is as yet no extensive simulation study to address these questions. We conduct such a study, including two computationally demanding structured association methods, as well as the simple and popular genomic control approach. We find that all these methods perform reasonably well although each has limitations that we illuminate. However our most important finding is that simple and computationally fast statistical procedures based on logistic regression - readily available in many statistical software packages - is superior to the tailor-made software that we consider. This is surprising since logistic regression does not explicitly model the population structure. It seems that including unlinked markers as predictors in a logistic regression analysis automatically adjusts for population structure: each marker soaks up some of the spurious association due to population stratification. Our conclusion is that even very large well-designed association studies can be conducted without fear of substantial numbers of false positives due to population structure, using only well-known and routinely-available statistical analysis methods.

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False Discovery or Missed Discovery? Using Linkage to Improve Power of Association Tests. *K. Roeder[1], L. Wasserman[1], S-A. Bacanu[2], B. Devlin[2].* 1) Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA; 2) Dept Psychiatry, Univ. of Pittsburgh, PA.

To scan the genome for association between markers and complex disease typically requires testing hundreds of thousands of genetic polymorphisms. Testing such a large number of hypotheses exacerbates the tradeoff between power to detect meaningful associations and the chance of making false discoveries. Although the full genome is scanned, investigators typically favor certain regions of the genome based on the results of prior investigations. The remaining regions of the genome are investigated simultaneously because genotyping is relatively inexpensive compared to the cost of recruiting participants in a genetic study, and because prior evidence is rarely sufficient to rule out these regions as harboring liability genes. Moreover technological advances permit inexpensive testing of a predetermined set of markers. However, this broad genomic investigation diminishes power to detect association, even for genes falling in regions of the genome favored a priori. Multiple testing problems of this nature are well suited for application of the false discovery rate (FDR) principle, which can improve power. To further enhance power, a new FDR approach is investigated that involves weighting the hypotheses based on prior data. We present a method for using linkage data to weight the association p-values. Our investigations reveal that if the linkage study is informative, the procedure improves power considerably. Remarkably, the loss in power is small even when the linkage study is uninformative. For the space of genetic models likely to underlie complex phenotypes, we calculate the sample size required to obtain useful prior information from a linkage study. Next we find the sample size required to obtain sufficient power to warrant a genome scan association study, assuming various levels of prior information obtained from a previously conducted linkage study. This inquiry reveals that among genetic models that are seemingly equal in genetic information, some are much more promising than others for this mode of analysis.

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The power of genome-wide association studies. *R.J. Klein, J. Ott.* Laboratory of Statistical Genetics, Rockefeller University, New York, NY.

Recent technological advances have made the high-throughput genotyping of a large number of SNPs possible. This has opened the door to genome-wide association studies, in which a collection of SNPs spanning the genome are typed to find those variants associated with a disease. These studies do not genotype all potentially causative SNPs. Instead, they aim to genotype a subset of marker SNPs that, because of linkage disequilibrium (LD), will allow detection of a causative variant that is not genotyped. Several products are or will soon be on the market for genotyping large numbers of SNPs simultaneously. One question that has not been answered is how one can estimate the power of a genome-wide association study. Given genotype frequencies in cases and controls and the number of cases and controls, calculation of the power of an association study is straightforward. This is true even for a large number of genotyped SNPs, assuming the causative SNP is in the set that is genotyped, because the significance level can be adjusted by the Bonferroni correction. To account for an ungenotyped functional mutation in LD, we need to know the extent of LD in the population between the ungenotyped mutation and the marker variant. Then, the power of detecting an association at the ungenotyped mutation using the marker can be computed. The overall power is then simply the average power over all the possible SNPs. Using this approach and data from the International HapMap project, we show that for the Affymetrix 100K genotyping platform and reasonable effect and sample sizes, power is highest if a study is performed in a population from Utah of northern and western European ancestry. Studies of Han Chinese from Beijing or Japanese from Tokyo would have intermediate and equal power, while studies of Yoruba in Ibadan, Nigeria would have the least power. For a risk allele with a population frequency of around 6% and a two-fold relative risk, approximately 3000 cases and 3000 controls would be needed to get a power of over 50%. In contrast, higher power was achieved using a set of only 44,000 SNPs selected to maximize the power of the study. This suggests that SNP selection is key to the power of a genome-wide association study.

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Combining correlated p-values in trait-SNP association studies. *K.N. Conneely, M. Boehnke.* Dept Biostatistics, University of Michigan, Ann Arbor, MI.

Whole-genome association studies will yield an abundance of data for localizing and identifying genetic variants that predispose to complex human diseases. For many such studies, each SNP marker will be tested for multiple phenotypes, resulting in a large array of tests. Since this creates vast amounts of information to process, it may be convenient to combine the information from these multiple correlated tests into a single overall test for each SNP or group of SNPs. Assessing overall significance is difficult in this situation due to the correlation between tests, since markers may be in linkage disequilibrium (LD) and traits may be correlated. Most corrections for multiple testing are only valid for independent tests. For example, Fishers (1932) method, which compares the summed log p-values to a chi-square distribution to obtain a global p-value, is anti-conservative when tests are correlated. A common solution is to use methods such as Fishers, but to rely on permutation tests rather than analytical distributions to estimate a global p-value. If permutation maintains the original correlation structure, such tests are unbiased but often not very powerful. A potentially more powerful approach, suggested by Zaykin et al. (2002), is to decorrelate the p-values by pre-multiplying a normalized vector of p-values by the Cholesky factor of the vectors correlation matrix. This approach produces a set of independent p-values which may then be combined using Fishers method or other similar methods. Decorrelating the p-values requires knowledge of the correlation matrix of the test statistics. We propose a method (and software in R) for estimating this matrix from the data using the residuals and slope estimates from the trait association test and the LD between the markers. We simulate data under a variety of models to assess the validity and power of this method to detect association between SNPs in LD and correlated traits. Decorrelating the p-values generally increases power substantially relative to 1) ignoring the correlation and 2) other procedures for handling multiple correlated tests. Our results suggest that this method is a powerful and computationally highly efficient tool for analyzing the wealth of whole-genome data.

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Quantifying the relationship between linkage disequilibrium (LD) and association tests: how correlated are test statistics if LD between SNPs is high? *D. Nielsen.* Statistics, North Carolina State Univ, Raleigh, NC.

One of the goals of the HapMap project is to provide information that will allow investigators to select a small subset of SNPs from a larger set so that genotyping costs of association mapping studies can be reduced, presumably without a substantial loss of power. Many different selection methods have been proposed to accomplish this. These include strategies that utilze information on haplotype blocks as well as methods that rely instead on patterns of linkage disequilibrium (LD) that do not assume strict block boundaries. The assumption behind these SNP selection strategies is that there is an advantageous relationship connecting LD between SNPs to the power to detect association with these SNPs. We have examined this relationship directly. Haplotypes were simulated for pairs of SNPs in a way that reflected patterns of LD estimated from real data. These haplotypes, together with a phenotype, were assigned to individuals so that no association between the two was expected. Each SNP in a pair was then evaluated using a standard case-control association test. This was repeated 10,000 times for each SNP pair, and the results used to estimate the degree of correlation between the two tests. Using this approach, estimates of the correlation between the tests could be compared with estimates of LD between the two SNPs. By plotting these two estimates for each of the more than 35,000 SNP pairs that were examined, a tight connection between the correlation of the tests and LD becomes evident. This relationship is, however, not linear. Instead, LD must get quite high before correlation between the tests becomes substantial. For example, correlation between the tests reached about 0.3 only when LD correlation coefficient, r^2, reaches about 0.8. We extend these analyses to family based association tests and to tests of quantitative traits. We also consider relationships between tests when associations between the SNPs and the phenotype exist.

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Long tracts of homozygosity in the HAPMAP data. *J. Gibson, N.E. Morton, A.R. Collins.* Human Genetics, University of Southampton. SO16 6YD. UK.

An individual is homozygous for a marker if the two alleles at that marker are identical. In some individuals long tracts of homozygosity can be found where homozygous markers occur in an uninterrupted sequence. The most obvious explanation for such tracts is autozygosity, where the same chromosomal segment has been passed to a child from parents who both inherited it from a common ancestor. Assuming recombination breaks up long chromosome segments over time, the length of a homozygous segment should be related to the time since the last common ancestor of the parents. In an inbred population we would expect to see longer segments of homozygosity than in outbred populations. However, using SNP genotypes from the apparently outbred populations in the HAPMAP data we have found segments of several megabases that appear to be identical by descent. Long segments of homozygosity have been recorded in the CEPH population (Broman & Weber, 1999), but this was in part due to an identifiable relationship between some pedigrees. The dense SNP typing of the HAPMAP project provides a unique opportunity to establish the distribution of size and location of these segments and their relationship to linkage disequilibrium patterns. The longest homozygous tracts we have determined to date span 11Mb in a Yoruban individual and 6.5Mb in a CEPH individual. Many factors may influence their length, abundance and location including SNP mutation rate, population structure, uniparental disomy, natural selection, recombination, and linkage disequilibrium patterns. We have examined the relationship between the location and size of tracts of homozygosity and the linkage disequilibrium structure as represented in linkage disequilibrium unit (LDU) maps. The correlation between the regional LDU/Mb and tract length is -0.17 (.0001), suggesting that the longer tracts are found in regions of strong LD. Our preliminary studies show that homozygous segments are surprisingly common and long in unrelated individuals from the outbred populations represented in HAPMAP. Their presence is likely to be associated with both autozygosity and regions of extensive LD, and have substantial impact on association mapping studies. Am. J. Hum.Genet. 65:1493-1500, 1999.

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Potassium channels and neurodegeneration: mutations in KCNC3 cause Spinocerebellar ataxia type 13 (SCA13).

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Potassium channel mutations have been described in episodic neurological diseases, though not late-onset degenerative disorders. We identified a Filipino pedigree with adult onset gait/limb ataxia, dysmetria, hypotonia, dysarthria, and nystagmus. Linkage analysis mapped the causative mutation to a ~4cM region of 19q13. This partially overlapped with the previously mapped 11.4cM SCA13 locus described in a French pedigree with childhood onset mental retardation, and relatively pure cerebellar ataxia. Both pedigrees show marked cerebellar atrophy on MR imaging. Using STR markers we narrowed the candidate region to a ~900kb region which contained the KCNC3 channel. Sequence analysis of KCNC3 revealed two missense mutations in exon 2: 1554GA (R420H) in the Filipino pedigree and 1639CA (F488L) in the French pedigree. No other sequence variants were observed. KCNC3 (Kv3.3) is a fast-rectifying voltage-gated Shaw subtype potassium channel with enriched expression in the cerebellum. The mutations occur in functionally critical regions of the protein and alter highly conserved residues. Screening of two hundred alleles from normal individuals revealed no polymorphisms at either site. We expressed wildtype and the R420H mutant KCNC3 in X. laevis oocytes. Expression of the wildtype channel resulted in currents with fast activation kinetics and a depolarized range of activation, as expected. In contrast, the 1554GA mutant showed no detectable channel activity. Co-expression of wildtype and mutant KCNC3 resulted in decreased current amplitude consistent with a dominant negative effect of the mutant allele. Identification of KCNC3 mutations contributes a new class of genes to causative etiologies of progressive degenerative ataxias. These findings highlight the importance of voltage-gated potassium channels in both neuronal function and cell survival and should lead to their investigation in other neurodegenerative diseases.

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A mutation in the COL9A1 gene in a family with a new autosomal recessively inherited form of Stickler syndrome. *S. Usami[1], R. Snoeckx[2], J. Van den Ende[2], F. Declau[3], E. Smets[4], F. Vanhoenacker[5], P. Van de Heyning[3], G. Van Camp[2].* 1) Dept Otorhinolaryngology, Shinshu Univ, Matsumoto, Naga, Japan; 2) Dept. of Medical Genetics, University of Antwerp; 3) Dept. of Otorhinolaryngology, University of Antwerp; 4) Dept. of Ophthalmology, University of Antwerp; 5) Dept. of Radiology, University of Antwerp.

Stickler syndrome is characterized by ophthalmic, articular, orofacial and auditory manifestations. It has an autosomal dominant inheritance pattern and is caused by mutations in COL2A1 (MIM 108300), COL11A1 (MIM 604841) and COL11A2 (MIM 184840). We describe family of Moroccan origin, comprising 4 children with Stickler syndrome, 6 unaffected children and 2 unaffected parents that were distant relatives (fifth degree). Four children showed symptoms characteristic of Stickler syndrome, including moderate to severe sensorineural hearing loss, high myopia with vitreoretinopathy and epiphyseal dysplasia. We considered the COL9A1 gene as a candidate gene on the basis of the structural association with type II and XI collagen and because of the high expression in the human eye and inner ear indicated by cDNA microarray. In addition, COL9A1 shows an expression pattern in cartilage, eye and cochlea that is very similar to type II, IX and XI collagens. Mutation analysis of the coding region of the COL9A1 gene showed a homozygous R295X mutation in the 4 affected children. The parents and 4 children were heterozygous carrier of the R295X mutation. Two children were homozygous for the wildtype allele. None of the family members except for the homozyogous R295X carriers had any signs pointing to Stickler syndrome. COL9A1 is the fourth gene that can cause Stickler syndrome. In contrast to the three previous Stickler-causing genes, this form of Stickler syndrome has an autosomal recessive inheritance pattern. This finding has a major impact on the genetic counselling of Stickler syndrome patients and on the understanding of the pathophysiology of collagens. Mutation analysis of this gene is recommended in Stickler patients with unaffected parents or Stickler patients that have no mutations in COL2A1, COL11A1 and COL11A2.

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A novel class of PAR1 deletions excluding *SHOX* is associated with Léri-Weill dyschondrosteosis (LWD). *K.E. Heath[1], S. Benito-Sanz[1], N.S Thomas[2], C. Huber[3], D. Gorbenko del Blanco[1], M. Aza-Carmona[1], J. Crolla[2], V. Maloney[2], V. Cormier-Daire[3], A. Campos-Barros[1], J. Argente[1].* 1) Dept. Endocrinology, Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, Madrid, Spain; 2) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK; 3) Dept. of Genetics and INSERM U393, Hospital Necker Enfants Malades, Paris, France.

Léri-Weill dyschondrosteosis (LWD) is a pseudoautosomal dominant skeletal dysplasia characterized by disproportionate short stature and Madelung deformity. Mutations in *SHOX*, located in the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes have been associated with LWD, Langer mesomelic dysplasia (LMD) and idiopathic short stature (ISS). Defects in *SHOX* have been identified in 60% of LWD cases while in the remaining 40% the molecular basis is unknown. This suggests genetic heterogeneity or the presence of mutations in unanalyzed *SHOX* regions such as the upstream, intragenic or downstream regulatory sequences.

Therefore, using a novel panel of microsatellites and SNPs, we analyzed the PAR1 sequences flanking *SHOX*, in 80 LWD patients in whom no *SHOX* deletion/mutation had been detected.

We identified the presence of a novel class of PAR1 deletions that exclude the *SHOX* locus, in 12 LWD patients (i.e. 15% of LWD probands). The deletions were of variable size and mapped between 30-530kb 3'downstream of *SHOX*. All shared a common deleted region of 29kb. Cosegregation with the phenotype was observed in all cases. Deletions were confirmed by FISH in 4 analyzed families. The presence of PAR1 deletions downstream of *SHOX* in LWD patients suggest two alternative hypotheses: a position effect due to distal regulatory elements of *SHOX* transcription in PAR1 or, alternatively, the existence of an additional locus apparently involved in the pathogenesis of LWD. Deletion analysis of this newly identified region should be included in the mutation screening of LWD, LMD and ISS patients. (kheath71@yahoo.com).

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Mutation in Non-Classic Region of the *p63* Gene causes Hay-Wells Syndrome. *T.A. Maher[1], G. Zhao[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Hay-Wells syndrome or Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC syndrome) (OMIM 106260) is a rare autosomal dominant disorder with variable expressivity. Clinical features include congenital filiform eyelid fusion, ectodermal dysplasia, cleft lip/palate, hypoplastic maxilla and deafness. Several features overlap other ectodermal dysplasia syndromes including ectrodactyly,ectodermal dysplasia, and facial clefting (EEC;OMIM 604292), split hand-split foot malformation (SHFM;OMIM 183600), limb-mammary syndrome (LMS;OMIM 603543), acro-dermato-ungual-lacrimal-tooth syndrome (ADULT;OMIM 103285) and cleft lip/palate, ectodermal dysplasia syndrome (CLPED1;OMIM 225060). Mutations have been described in the *p63* gene for these ectodermal dysplasias and genotype-phenotype correlations have been recognized for EEC and AEC syndromes. The vast majority of mutations resulting in AEC have been reported to be found in the sterile alpha motif (SAM) domain of the *p63* gene (mostly exon 13). We report a 30 year old Puerto Rican male with classic findings of AEC syndrome. Bidirectional sequencing of the *p63* gene using an ABI 3100 detected a missense mutation (T193K) in exon 4. The mutation was confirmed using restriction digestion and is in the DNA binding domain of the *p63* gene, typically associated with ADULT, EEC, and SHFM phenotypes. Hence, mutation screening in individuals with classic AEC should start with sequencing of the exons coding for the SAM domain and continue if negative to the entire gene. To our knowledge, this is the first report of a mutation in the DNA binding domain of the *p63* gene causing AEC.

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LADD syndrome is caused by *FGF10* mutations and is allelic to autosomal dominant Aplasia/Hypoplasia of Lacrimal and Salivary Glands. *J.M. Milunsky[1,2,3], G. Zhao[1], T.A. Maher[1], D.B. Everman[4].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA; 4) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC.

Lacrimo-auriculo-dental-digital syndrome (LADD;OMIM 149730) is an autosomal dominant MCA disorder characterized by aplasias, atresias or hypoplasias of the lacrimal/salivary system, cup-shaped ears and hearing loss, in addition to dental and digital anomalies. Loss of function mutations in the *FGF10* gene were recently described (Entesarian et al, 2005; Milunsky et al, 2005) resulting in aplasia of lacrimal and salivary glands (ALSG; OMIM 180920 and 103420). Due to the significant phenotypic overlap and variable expressivity, we hypothesized that the LADD syndrome may be an allelic disorder. Our proband is a three year old female with nasolacrimal fistulae, dacryostenosis, bilateral atresia of the lacrimal system, hypoplastic biphalangeal thumbs, dental anomalies, right esotropia, and small prominent ears in whom a diagnosis of LADD syndrome was made following an extensive negative work-up. Parents are non-consanguineous and unaffected. Bidirectional sequencing of the coding regions of the *FGF10* gene using an ABI 3100 in our proband demonstrated a single nucleotide substitution in exon 3 (I156R) resulting in a non-conservative amino acid change from isoleucine to arginine. This missense mutation was confirmed by restriction enzyme digestion and was not found in 250 normal controls. Parental studies are anticipated in the near future. Expression studies of *FGF10* have previously demonstrated its presence and when disrupted, its effects in all of the systems with anomalies in LADD syndrome including lacrimal, inner ear, dental, limb, midface and genitourinary. Further studies are ongoing to determine if genotype/phenotype correlations exist. We have now shown that haploinsufficiency of the *FGF10* gene leads to ALSG and a missense mutation results in LADD syndrome, providing evidence that ALSG and LADD syndrome are allelic disorders.

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Freeman-Sheldon syndrome is caused by defects of myosin. *R. Toydemir[1], D. Stevenson[2], L.B. Jorde[1], J. Carey[2], M. Bamshad[1,2].* 1) Dept Human Genetics, Univ Utah, Salt Lake City, UT; 2) Dept Pediatrics, Univ Utah, Salt Lake City, UT.

Several years ago, we discovered that missense mutations in *TPM2* encoding tropomyosin cause distal arthrogryposis type 1, while missense mutations in either *TNNI2* or *TNNT3*, encoding isoforms of troponin I (TnI) and troponin T (TnT) respectively, cause Sheldon-Hall syndrome (SHS). Tropomyosin, TnI, and TnT are components of the troponin-tropomyosin complex of fast-twitch myofibers and are expressed primarily in the peripheral muscles of the limbs. Based on these observations we hypothesized that other DAs were caused by mutations in genes encoding other structural components of the contractile apparatus of fast-twitch myofibers. We have now screened several additional genes encoding proteins of the contractile machinery of fast-twitch myofibers and identified new mutations causing other distal arthrogryposis syndromes, most notably classical Freeman-Sheldon syndrome (FSS).

FSS is a rare, multiple congenital contracture syndrome that is nonetheless, relatively well known because affected children have a striking appearance-it was historically called whistling-face syndrome because of involvement of the facial muscles. We screened 20 probands with FSS and found 4 different mutations a myosin heavy chain gene (*MYH*) in 19 cases (95%). Most *MYH* mutations caused non-synonymous amino acid substitutions, and one specific codon was mutated in 14 (70%) of cases. Mutations in *MYH* were also found in 9 cases (~25%) of SHS. None of these mutations were found in unaffected family members or 450 control chromosomes from an ethnically matched population. Each of the affected codons is strongly conserved among *MYH* orthologs and paralogs. No substantial relationship between the location of a mutation and the clinical characteristics of either FSS or SHS was observed. Additionally, three mutations in *MYH* were shared between FSS and SHS cases suggesting that the phenotype of both disorders is influenced by other genetic factors and/or environmental factors. Studies are underway to explore the mechanism by which mutations in *MYH* cause congenital contractures in FSS and SHS.

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Familial Soft Tissue Cavernous Hemangioma (FSTCH): A new autosomal dominant disorder. *T.C. Falik-Zaccai[1,2], E. Blechman[3], M. Khayat[1], M. Genser[1,2], L. Even[2,3], I. Cohen[4], N. Heno[2,3].* 1) Inst. of Medical Genetics, Western Galilee Hosp, Nahariya, Israel; 2) Rappaport faculty of Medicine, Technion, Haifa, Israel; 3) Dep. of Pediatrics, Western Galilee Hosp, Nahariya, Israel; 4) Dep. of Pathology, Western Galilee Hosp, Nahariya, Israel.

Cavernous hemangioma is a benign vascular tumor of dilated blood vessels mostly capillaries or veins filled with blood. A familial form of cavernous hemangioma of the CNS has been well described. We report here a new phenotype of FSTCH that occur in different organs and systems such as skin, subcutaneous tissues, muscles, and oral or lingual mucus membranes without infiltration into the CNS or viscera. FSTCH was diagnosed in fifty two affected individuals out of 250 members of a large 7 generations Druze kindred related to a single ancestor. The lesions start to appear within a few months after birth and their number increase with age. The lesions cause pain, discomfort and significant self esteem problems but do not affect mortality. Surgery of lesions is of limited benefit due to recurrent growth. The pedigree structure and statistical analysis support autosomal dominant inheritance of FSTCH in this family. Linkage analysis to five loci, mapped to chromosomes 3, 5 and 7, that are known to be involved in familial cerebral cavernous hemangioma / malformation was performed. Linkage to these loci was ruled out, thus, indicating that a novel locus is involved in FSTCH in the present family. The structure of the pedigree and number of individuals in this family enable us to map and identify the FSTCH causative gene. Studies are in progress. This is the first report of a new (non CNS) cavernous hemangioma disoder. Identification of the causative gene and its protein product might shed light on important new pathways playing a significant role in the normal biology of angiogenesis.

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***RAI1* mutation in Smith-Magenis syndrome.** *S. Girirajan[1], D.J. Bunyan[3], S.H. Elsea[1, 2].* 1) Dept. of Human Genetics; 2) Dept. of Pediatrics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA; 3) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire, United Kingdom.

Smith-Magenis syndrome (SMS) (OMIM #182290) is a mental retardation syndrome characterized by distinctive craniofacial features, delayed milestones, cognitive impairment and mild to severe mental retardation. Behavioral abnormalities include significant sleep disturbance and maladaptive and self-injurious behaviors. SMS is caused by either deletion or mutation of the *RAI1* gene located in the Smith-Magenis syndrome region on 17p11.2. We have analyzed 60 putative SMS patients referred to us for molecular evaluation of 17p11.2. FISH using an *RAI1*-specific probe revealed a heterozygous 17p11.2 deletion in 23/60 patients. The remaining 37 patients were further evaluated for mutations in the coding region of *RAI1* using a PCR-based sequencing strategy. In this study, we identified novel mutations in *RAI1* in 9/37 (24%) individuals. Mutations include small and large intragenic deletions and missense mutations. We also identified several polymorphisms that are new and unclassified, as well as those that are reported in the NCBI SNP database. Clinical analyses of these patients suggest certain differences between the 17p11.2 deletion patients and those with mutations in *RAI1*; however, the neurobehavioral anomalies unique to SMS are consistent between the two groups. Notable are absence of visceral abnormalities, failure to thrive, iris anomalies, and cleft lip and palate. Further, these individuals show decreased incidence of short stature, hearing loss, motor delay and hypotonia compared to those with 17p11.2 deletions. These data suggest that mutations in *RAI1* contribute a substantial portion to the causation of SMS; thus, putative SMS patients with no deletion of 17p11.2 should be evaluated for mutations in *RAI1*.

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Williams Syndrome: Gene expression is related parental origin and regional coordinate control. *J.C. Collette[1], K. Tea[1], U. Bellugi[2], A. Galaburda[3], D.L. Mills[4], A.L. Reiss[5], J.R. Korenberg[1].* 1) Neurogenetics, Cedars-Sinai Med Ctr, Los Angeles, CA; 2) Salk Inst for Biological Sciences, La Jolla, CA; 3) Beth Israel Deaconess Med Ctr, Boston, MA; 4) Univ of California, San Diego, CA; 5) Stanford Univ School of Medicine, Stanford, CA.

Williams syndrome is a neurodevelopmental disorder caused by a deletion of chromosome 7q11.23 including about 24 genes. We have shown that the deletion of genes encoded by GTF2I and GTF2IRD1 was related to WS features and now provide evidence that WS gene expression from the non-deleted 7 is related to parental origin of the deletion and regional transcriptional control. We hypothesize that this variation contributes to WS features, full scale IQ, visual spatial deficits, social-emotional, linguistic and neuroanatomic variations. To elucidate the underlying genetic and neural mechanisms, we have examined WS gene expression in a cohort of 99 individuals with WS (41 males aged 28.1 10.9 years, 58 females aged 26.5 12.2 years) and 48 controls (24 females, 24 males). We established the regions deleted and gene expression in lymphoblastoid cell lines using TaqMan-based quantitative real-time PCR and probes for TBL2, GTF2IRD1, GTF2I, NCF1 (gene and pseudogenes) and WBSCR16, and controls from the non -WS regions of chromosome 7. Using b actin as a control, we found: 1. GTF2I expression was decreased in WS versus controls, ($p < 0.0001$); 2. GTF2I and TBL2 expression was higher in WS subjects with deletions derived from the father than in those whose deletions derived from the mother ($p < 0.05$; $p < 0.05$), in contrast to NCF1 expression of which was higher in subjects with maternal than in paternal deletions ($p < 0.05$); 3. Expression GTF2I was directly proportional to TBL2; more in WS versus controls ($p < 0.0001$ in WS, $p < 0.002$ in controls), and to GTF2IRD1 ($p < 0.002$) as well as to WBSCR16 ($p < 0.0001$) and to NCF1 ($p < 0.0001$). These data support the hypothesis that gene expression and possibly phenotype in WS are predicted by parental origin of the deletion and local transcriptional control. The use of WS to parse the contribution of genetic mechanisms to human behavior links these to the elusive systems neurobiology of humans.

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The 9q subtelomere deletion syndrome: Further delineation of the common breakpoint and critical region and analysis of Eu-HMTase1 as a candidate gene for congenital heart disease. *C. DeScipio[1], M.T. Saung[1], M. Kaur[1], D.R. Stewart[2], E. Goldmuntz[1], N.B. Spinner[1], H. Riethman[3], I.D. Krantz[1].* 1) Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA. and The University of Pennsylvania School of Medicine, Philadelphia, PA; 2) NHGR/NIH, Bethesda, MD; 3) Wistar Institute, The University of Pennsylvania School of Medicine, Philadelphia, PA.

The 9q subtelomere deletion syndrome consists of several common phenotypic findings including mental retardation, distinct facial features, and congenital heart defects (primarily conotruncal defects). Molecular characterization of the breakpoint in a cohort of twelve individuals defined an ~1.2 Mb commonly deleted region containing 14 genes. In the majority of individuals, the proximal breakpoint clustered to within a 400kb interval, suggesting either a genomic predisposition to rearrangement or proximal genes that may result in embryonic lethality. Kleefstra et al. (J Med Genet 2005) recently reported an individual with features of the 9q subtelomere deletion syndrome and a balanced de novo t(X;9)(p11.23;q34.3) which was shown to disrupt a gene (euchromatin histone methyl transferase1, Eu-HMTase1), that maps to within our defined minimal commonly deleted region. This identified Eu-HMTase1 as a candidate gene for some or all of the features of the 9q subtelomere deletion syndrome. Since congenital heart defects are present in ~50% of individuals with the 9q subtelomere deletion syndrome, as well as in the patient described by Kleefstra et al., the Eu-HMTase1gene, which is expressed developmentally in the heart, may have a role in cardiac development. Using conformation sensitive gel electrophorsis (CSGE), we are completing mutational analysis in 150 individuals with isolated congenital heart defects (50 with ventricular septal defects (VSD), 50 with atrial septal defects (ASD), and 50 with Tetralogy of Fallot (TOF)). All individuals in this cohort had a normal karyotype and 22q11 fluorescence in situ analysis (FISH). Further genomic characterization of the common breakpoint region and results of mutational analysis of Eu-HMTase1 will be presented.

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Enzyme replacement therapy (ERT) with recombinant human arylsulfatase B (rhASB) for MPS VI (Maroteaux-Lamy): 96 week follow-up. *P. Harmatz[1], D. Ketteridge[2], J. Hopwood[2], R. Giugliani[3], I. Schwartz[3], N. Guffon[4], C. Sa Miranda[5], E. Teles[5], J.E. Wraith[6], M. Beck[7], M. Scarpa[8], Z-F. Yu*[9], J. Wittes*[9], S.J. Swiedler**[9].* 1) Children's Hospital, Oakland, CA; 2) LDRU, Adelaide; 3) Med Genet Serv HCPA, Brazil; 4) Hosp Edouardo Herriot Pavillon, Lyon, France; 5) Hosp de Sao Joao and IBMC, Porto, Portugal; 6) RMCH, Manchester, UK; 7) Children's Hosp, U of Mainz, Germany; 8) Pediatrics, U of Padova, Italy; 9) *Statistics Collaborative, Inc, Washington, D.C.; **BioMarin Pharmaceutical Inc, Novato, CA.

MPS VI is a rare, life-threatening lysosomal storage disease. ERT has shown positive results in 3 clinical studies and received FDA approval as galsulfase (Naglazyme). Efficacy and safety are reported through 96 wks. Patients were enrolled in 1 of 3 studies: Phase 1 / 2 (2 dose, blinded x 6 mo, then open-label at 1mg/kg), Phase 2 (open-label, 1mg/kg), or Phase 3 (randomized, double-blind, placebo-cont x 6 mo at 1 mg/kg, then open-label). Endpoints included 6 or 12 min walk tests (6MWT or 12MWT), 3 min stair climb (3MSC), urine glycosaminoglycans (GAGs), adverse event monitoring, safety labs, and anti-rhASB antibody. Fifty-five patients were enrolled; 53 continue to receive ERT. Data are reported for Phase 1 / 2(n=5) and Phase 2(n=10) through 96 wks. Phase 3 data through 96 wks will be presented at the meeting. Phase 1 /2 patients receiving rhASB for 96 wks improved 99m in the 6MWT (mean, p=0.046, Wilcoxon Signed-Rank), Phase 2 patients improved 246m in the 12MWT (mean,p=0.005). Similar improvements in 3MSC were noted with Phase 2 patients increasing from 50 to 114 stairs (mean,p=0.005). All patients had rapid, persistent decline in urinary GAGs by mean 75%. Improvement in height (mean 3.9 cm, p=0.004) and forced vital capacity (FVC, mean 0.1 L, 17%, p=0.001) occurred in most patients. Infusions were generally well tolerated. All but one patient developed anti-rhASB antibody, but these antibodies were not associated with infusion-associated reactions or lack of clinical benefit. In conclusion, rhASB maintains improvement in physical mobility, endurance, and urine GAGs over 96 wks, and has an acceptable safety profile.

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The phase II/III I2S enzyme replacement (ERT) clinical trial results for MPS II. *J. Muenzer[1], E. Wraith[2], M. Beck[3], R. Giugliani[4], P. Harmatz[5], C.M. Eng[6], A. Vellodi[7], R. Martin[8], U. Ramaswami[9], M. Calikoglu[1], S. Vijayaraghavan[2], A. Puga[4], B. Ulbrich[9], M. Shinawi[6], M. Cleary[7], S. Wendt[3].* 1) Univ North Carolina, Chapel Hill, NC; 2) Royal Manchester Childrens Hospital, Manchester, UK; 3) Univ of Mainz, Mainz, Germany; 4) Medical Genetics Service, HCPA/UFRGS, Brazil; 5) Children's Hospital, Oakland, CA; 6) Baylor College of Medicine, Houston, TX; 7) Great Ormond Street Hospital, London, UK; 8) St. Louis Childrens Hospital, St Louis, MO; 9) Cambridge Univ Teaching Hospitals, Cambridge, UK.

MPS II (Hunter syndrome) is caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S). Currently there is no effective therapy for MPS II. ERT with I2S was evaluated in a multicenter clinical trial in MPS II patients. The main objective of this trial was to determine the efficacy of I2S (0.5 mg/kg) given weekly or biweekly versus placebo over 1 year. The primary efficacy endpoint was the change from baseline to wk 53 in a 2-component composite score using forced vital capacity (FVC) and distance walked in 6 min. Of the 96 enrolled patients, 94 completed the study and all have elected to participate in an open-label extension study. Treatment with I2S was well-tolerated and the most common adverse events observed were associated with the clinical manifestations of the disease. Of the adverse events considered possibly related to I2S, infusion related reactions were the most common and generally mild. The mean improvement from baseline to wk 53 in % predicted FVC was 3.4% in patients receiving I2S compared to 0.8% for placebo patients. The mean increase from baseline to wk 53 in the distance walked by I2S treated patients was 44 meters as compared to 7 meters by the placebo group. Patients who received I2S showed a statistically significant improvement in the composite primary efficacy endpoint (p=0.0049) compared to placebo. Additional supportive efficacy data includes liver and spleen size, urine GAG and joint range of motion. These results indicate that I2S can be safely administered and is a promising treatment for MPS II.

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Enzyme Replacement Therapy (ERT) with Recombinant Human Acid Alpha Glucosidase (rhGAA) in Infantile-onset Pompe Disease: Interim Results from a Pivotal Trial. *P. Kishnani[1], B. Byrne[2], M. Nicolino[3], H. Mandel[4], N. Leslie[5], W.L. Hwu[6], E. Wraith[7].* 1) Department of Pediatrics, Duke University Medical Center, Durham, NC; 2) Shands Hospital at the University of Florida, Gainesville, FL; 3) Pediatrique Hopital Debrousse, Lyon, France; 4) Ramban Medical Center, Haifa, Israel; 5) Cincinnati Children's Hospital, Cincinnati, OH; 6) National Taiwan University Hospital; 7) Royal Manchester Hospital, Manchester, UK.

Introduction. Pompe disease is caused by a deficiency of the lysosomal enzyme acid alpha glucosidase (GAA). IOPD is characterized by severe, progressive cardiomyopathy, muscle weakness, respiratory insufficiency, and death usually by one year of age. **Methods.** This is an open-label, randomized, dose-ranging, multinational, multicenter study. 18 patients <6 months of age, with cardiomyopathy, and residual GAA activity of <1% in skin fibroblasts were enrolled. rhGAA derived from CHO cell culture is administered IV at 20 or 40 mg/kg qow. Efficacy end-points include survival, ventilator-free survival, decrease in LVMI, and changes in growth parameters and motor development scores. **Results.** 11 males and 7 females were enrolled; 7 patients (39%) are Caucasian, 4 (22%) Black, 3 (17%) Asian, 2 (11%) Hispanic, and 2 (11%) of Other ethnicity. Median age at first symptoms was 1.0 month (range: 0-5.5 months). Median age at first infusion was 5.3 months (range: 1.2-6.1). After 6 months of ERT, 18 patients (100%) are alive and 17 patients (94%) are free of invasive-ventilator support. 15 patients (83%) developed anti-rhGAA antibodies. 8 patients (44%) had infusion associated reactions, mostly mild to moderate in severity and managed symptomatically. **Conclusions.** rhGAA has been generally well tolerated. After 6 months of ERT, patients age ranges from 7.2 to 13.3 months. Data analyses of all above described end points are ongoing and will be presented. Results of earlier clinical trials of ERT administration to a small number of patients with IOPD have been promising. This trial provides additional information on the safety and efficacy of rhGAA to a larger cohort of IOPD patients at two different doses.

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Adult Hypophosphatasia Treated with Teriparatide. *C. Deal[1], S. Mumm[2,3], M.P. Whyte[2,3].* 1) Cleveland Clinic, Cleveland, OH; 2) Div Bone and Mineral Dis, Wash Univer Schl Med and; 3) Research Center, Shriners Hospital; St. Louis, MO.

We report the first case of hypophosphatasia (HPP) treated with the parathyroid hormone sequence, teriparatide (rhPTH 1-34, TPTD). HPP features low serum alkaline phosphatase (ALP) activity from deactivating mutation of the gene for the tissue nonspecific isoenzyme of ALP (TNSALP). Extracellular accumulation of inorganic pyrophosphate (PPi) impairs skeletal mineralization causing rickets in children and osteomalacia in adults. There is no established therapy. Adult HPP typically presents with poorly-healing metatarsal stress fractures (MTSF) and unhealing femoral pseudofractures (FPF). At age 54, our patient had a painful MTSF with delayed healing. Two non-healing MTSFs occurred 2 yrs later. A FPF ensued. Pain occurred at all fractures. Serum ALP ranged from 10-24 IU/L (40-150 nl), and bone-specific ALP was 6 IU/L (12-31 nl). Other biochemistries were unremarkable except for two further features of HPP: elevated serum phosphate (Pi) of 5.1 mg/dl and vitamin B6 concentration of 603 ug/L (18-175 nl). DXA of the spine and hip showed Z-scores of +2.9 and +1.5, respectively. Treatment began at age 56 with 20 mcg TPTD daily. After 2 mo, pain improved in the thigh and foot. After 4 mo, no fracture site was painful. X-rays showed significant healing after 4 mo in 2 MTSFs and delayed healing with exuberant callus in 1 MTSF (no callus was present 9 mo after MTSF prior to TPTD treatment). The FPF appeared completely healed after 9 mo. A marked reduction in chondrocalcinosis in the symphysis pubis was seen after 9 mo of TPTD. Sequential biochemistries showed improved serum ALP activity (max. 43 IU/L) and sustained increments in NTX and OC. Ours is the first report of HPP treated with TPTD. Complete pain relief at fracture sites occurred after 2 mo; MTSP and FPF healed. Increased ALP, NTX, and OC suggested improved bone remodeling. Little change in circulating vitamin B6 levels indicated a focal (skeletal) effect of TPTD treatment. TPTD may improve adult HPP by enhancing osteoblast production of TNSALP and/or causing phosphaturia (lowering, at mineralization sites, extracellular Pi levels which acts as a competitive inhibitor of TNSALP).

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AAV1-mediated Gene Therapy For LPL Deficiency. *C. Ross[1], J. Twisk[2], J. Rip[3], M. Nierman[3], P. Dijkhuizen[2], W. Hermens[3], J. Meulenberg[3], J. Kastelein[2], J. Kuivenhoven[2], M. Hayden[1].* 1) UBC, Vancouver, Canada; 2) AMT, Amsterdam, Netherlands; 3) AMC, Amsterdam, Netherlands.

Lipoprotein lipase (LPL) deficiency results in excessive plasma triglycerides (TG 1,000 mg/dl), abdominal pain, and occasionally life-threatening pancreatitis, but there is no adequate treatment for LPL deficiency. A single intramuscular (IM) administration of AAV1 vector, encoding the human LPL-S447X variant, results in complete, long-term normalization of dyslipidemia in LPL -/- mice. To determine the conditions for clinical application, varied doses of AAV1-LPL-S447X (1x10(11)-1x10(12) genome copies (gc)/kg) and varied numbers of IM injection sites (2-50) were tested in the large feline model of LPL deficiency. RESULTS: Administration of 5x1011 gc/kg completely resolved the visible plasma lipemia within 3-7 days, and plasma TG were reduced 99.9 percent to normal levels (8-19 mg/dl). Intermediate efficacy was achieved at 1x10(11) gc/kg. By varying the dose per site, linear LPL expression was demonstrated over a wide range of local doses in muscle (4x10(10)-1x10(12) gc/site). In LPL -/- cats, efficacy was transient, due to inhibitory anti-LPL antibodies blunting LPL expression. The level and duration of efficacy were improved with cyclophosphamide immunosuppression. The cause of the anti-hLPL response is related to species differences between human and feline LPL (88 percent identical), since LPL -/- cats treated with AAV1-LPL-S447X also develop antibodies. Efficacy and safety studies in other species have now permitted the initiation of a clinical trial in LPL deficient patients. Eight patients have been characterized for enrollment. TG levels varied greatly per patient (1,000-4,000 mg/dl), even on a stringent low-fat diet, and episodes of pancreatitis were observed. All patients had no LPL catalytic activity, but LPL protein levels of 19-103 percent of normal were present, thus reducing the risk of immune responses towards transgenic LPL. Patient myoblasts, isolated from muscle biopsies, secreted catalytically active LPL after infection with AAV1-LPL-S447X. These data supported the initiation of a clinical trial, for whic regulatory approval has now been granted.

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Pseudo-ochronosis due to a common SNP in the organic anion transporter MRP4 / ABCC4: Pharmacogenomic implications. *R. Kleta[1,2], C. Klein[1], P. Suwannarat[1], H. Stanescu[1], A. Jeong[1], H.A. Austin[3], G. Burckhardt[4], W.A. Gahl[1].* 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD, USA; 2) Office of Rare Diseases, OD, NIH; 3) NIDDK, NIH; 4) Vegegative Physiologie und Pathophysiologie, Goettingen, Germany.

Alkaptonuria is a disorder of tyrosine catabolism in which homogentisic acid (HGA) accumulates and destroys connective tissue, causing darkened cartilage (ochronosis), joint and cardiac valve deterioration. In our investigation of 73 alkaptonuria patients, we identified 4 young women with an ochronotic phenotype but normal HGA excretion. All had been treated with the tetracycline derivative minocycline. Tetracyclines are secreted into the urine by proximal tubular organic anion transporters. These transporters recognize a variety of drugs / xenobiotics, including p-aminohippurate (PAH), which serves as a reference ligand and whose clearance provides a measure of tubular anion secretion. We sequenced all coding exons of OAT1, OAT3, OAT4, and MRP4 in two of our 4 pseudo-ochronotic patients. Both exhibited a homozygous intronic acceptor splice site mutation in MRP4, IVS2-5T>C. cDNA generated from leucocyte RNA showed that this splice site mutation leads to partial skipping of exon 3, comprised of 121 bp. This out-of-frame deletion produces a premature stop codon (c.186-306del; p.G62fsX67). PAH clearance was abnormally low in the one patient we studied, proving the deleterious effect of this mutation on organic anion transport. The two other patients were homozygous or heterozygous for this mutation. We generated a restriction site in MRP4 to screen for IVS2-5T>C. In 136 Caucasian alleles we found 70% wildtype and 30 % mutated alleles. This common MRP4 SNP, IVS2-5T>C, essentially defines MRP4 as a luminal PAH exit mechanism. It can also influence drug metabolism. First, it possibly can impair secretion of minocycline and create a pseudo-ochronotic state mimicking alkaptonuria. Second, it could modulate the clinical severity of alkaptonuria, since HGA secretion depends upon intact organic anion transport. Examination of normal individuals for this common SNP could provide guidance for the appropriate use of a host of antibiotic and chemotherapeutic agents.

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Search for additional genetic determinants of warfarin dose and bleeding side effect. *R. McGinnis[1], M. Wadelius[2], L. Chen[1], A. Rane[3], P. Deloukas[1].* 1) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Department of Medical Sciences, Clinical Pharmacology, University Hospital, Uppsala, Sweden; 3) Clinical Pharmacology, Karolinska Institute, Campus Huddinge, Stockholm, Sweden.

Warfarin is an anticoagulant that is the most widely prescribed therapy for reducing thromboembolic events that often give rise to stroke, deep vein thrombosis, pulmonary embolism or serious coronary malfunctions. Clinical use of warfarin is made difficult by the wide interindividual variation in dose required to achieve the usual magnitude of anticoagulant effect (2.0-3.0 PT INR) and by a serious side effect (severe bleeding) in a minority of patients. Following the recent identification of the gene that encodes the warfarin drug target (vitamin K epoxide reductase complex 1 or VKORC1), we analysed a sample of 200 warfarin-prescribed Swedish patients and reported that SNPs in VKORC1 are highly associated ($p<0.0001$) with warfarin dose (Wadelius et al, Pharmacogenomics J. May 2005). Each of the three most predictive SNPs (rs9923231, rs9934438, rs2359612) accounts for approximately 30% of the variance in warfarin dose and, when considered together with the only other confirmed genetic factor (cytochrome P450 CYP2C9) and with non-genetic factors (weight, age, diagnosis, other medications), accounts for more than 50% of the variance in dose. To extend these VKORC1 results and identify additional genetic factors that influence warfarin dose or cause the severe bleeding side effect, we are typing the original sample and 1300 additional warfarin-prescribed Swedish patients (WARG study) for VKORC1 SNPs as well as 270 tagSNPs selected in 25 additional candidate genes located in metabolic (e.g. CYP 450) and blood clotting factor pathways. Based on multiple regression and case-control analyses, we will present results of testing individual SNPs and haplotypes for association with warfarin dose or cause of severe bleeding.

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Defective *in vivo* Hedgehog signaling in NSDHL deficient mice, a model for cholesterol biosynthesis disorders. *F. Jiang[1], D. Cunningham[1], G. Herman[1,2].* 1) Center for Human and Molecular Genetics, Columbus Children's Research Institute, Columbus, OH; 2) Department of Pediatrics, The Ohio State University.

The X-linked *Nsdhl* gene encodes a sterol dehydrogenase involved in cholesterol synthesis. Mutations in this gene cause the male lethal phenotypes in human CHILD syndrome and bare patches (*Bpa*) mice. Affected male mouse embryos for several *Bpa* alleles die in mid-gestation with a thinner, less vascularized fetal placental labyrinth. Due to preferential inactivation of the paternal X in most extraembryonic lineages in female mouse embryos, the primary defect resulting in male lethality must lie in cells of extraembryonic mesoderm that demonstrate random X-inactivation. We hypothesized that NSDHL deficiency is disturbing one or more signaling pathways primarily in extraembryonic mesoderm in the developing placenta. We initially examined the Hedgehog (HH) signaling pathway since (1) HH proteins are modified by cholesterol during their processing and (2) other researchers have demonstrated abnormal HH signaling in cells cultured from patients with selected other disorders of cholesterol synthesis. *Patched (Ptch)*, encoding the HH receptor, is upregulated by HH signals and thus acts as a sensitive indicator of HH signaling. Although expression of a HH protein in the mouse placenta has not been reported, we have now demonstrated clear expression of PTCH in allantoic mesoderm of wild type placentas using X-gal staining of transgenic mice carrying a *Ptch-lacZ* reporter. In RT-PCR and *in situ* hybridization experiments, we demonstrate that the signaling molecule is Indian hedgehog (IHH), produced by yolk sac endoderm cells that migrate into allantoic mesoderm around 10dpc. There is no expression of Sonic or Desert hedgehog in the early fetal placenta. Affected placentas from mutant *Bpa* male embryos demonstrate markedly decreased Ptch-lacZ staining and migration of *Ihh* expressing cells. These data strongly implicate the HH signaling pathway in the pathogenesis of the placental defects in NSDHL deficiency and further provide the first evidence for a role for HH signaling in the development of a functional mammalian placenta.

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The second family with AGAT deficiency (creatine biosynthesis defect): diagnosis, treatment and the first prenatal diagnosis. *K. Johnston[1], L. Plawner[1], L. Cooper[1], G.S. Salomons[2], N.M. Verhoeven[2], C. Jakobs[2], A.J. Barkovich[3].* 1) Kaiser Permanente, San Francisco, CA; 2) VU University Medical Center, Amsterdam, The Netherlands; 3) Department of Radiology, University of California, San Francisco, CA.

Deficiency of arginine:glycine amidinotransferase (AGAT), the first step in creatine biosynthesis, has been described in three related individuals. We describe a new unrelated 3-year-old girl. She showed delayed motor milestones from 4 months of age. At 14 months she had moderate hypotonia, developmental delay and failure to thrive. After demonstrating moderate generalized organic aciduria, she was found to have extremely low levels of guanidinoacetic acid in urine (<0.3 mmol/mol creatinine) and plasma (<0.05 uM/l), with low plasma creatine (2.3 uM/l). Single voxel proton magnetic resonance spectroscopy (MRS) of the brain showed absence of creatine. AGAT activity was not detectable in lymphoblasts. DNA sequence analysis of the AGAT gene revealed a homozygous mutation (IVS3+1G>T), which results in the skipping of exon 3 (r.289_484del196). The parents are carriers of this mutation. In a subsequent pregnancy of the family, DNA isolated from CVS was found to be homozygous for the IVS3+1G>T mutation. At age 16 months, Bayley Infant Development Scale (BIDS) showed functioning at 7 month level (43% of chronologic age). Oral creatine supplementation (400 mg/kg/d) was begun. Growth rate accelerated to the normal range. BIDS at 18 months showed developmental age of 10 months, a clear increase in developmental trajectory. Creatine dose was increased to 600 mg/kg/d at 20 months, and reduced to 400 mg/kg/d at 27 months. BIDS at 29 months showed Mental Scale at 23 months and Motor Scale at 25 months (80% and 86% of chronologic age). Partial recovery of cerebral creatine levels was demonstrated on MRS at 25 months of age. Brain MRS at 33 months of age revealed creatine/NAA ratio about 50-60% of normal, compared with age-matched contols. Presenting symptoms of AGAT deficiency (developmental delay and hypotonia) are non-specific. Early identification and treatment of AGAT deficiency may lead to improvement of developmental outcome.

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Structural, biochemical, and functional characterization of the OPA3 gene, defective in Type III 3-methylglutaconic aciduria. *M. Huizing[1], L. Ly[1], H. Dorward[1], I. Nouvel[1], R. Kleta[1], B. Feldman[1], W.A. Gahl[1], Y. Anikster[2].* 1) MGB, NHGRI, NIH, Bethesda, MD; 2) Sheba Medical Center, Israel.

Type III 3-methylglutaconic aciduria is a neuro-ophthalmologic syndrome of early-onset bilateral optic atrophy and later-onset spasticity, and extrapyramidal dysfunction. Urinary excretion of 3-methylglutaconic acid (3MGA) and of 3-methylglutaric acid is markedly increased. In 2001, we identified the causative gene, *OPA3*, consisting of two exons coding for a 179-amino acid protein. Here we report that *OPA3* also produces a novel transcript consisting of the common exon 1 spliced directly to a 3rd exon, skipping exon 2 altogether. This transcript is conserved in other species, and exon 3 closely resembles exon 2, suggesting a common evolutionary origin. Molecular analysis of 11 patients with unexplained optic atrophy and increased urinary 3MGA did not reveal exon 3 mutations. The OPA3 protein, whose function remains unknown, contains an N-terminal mitochondrial leader sequence and targeting signal and a putative C-terminal peroxisomal targeting signal. Since increased 3MGA levels can result from defects in either the mitochondrion (e.g., leucine degradation) or the peroxisome (e.g., mevalonate shunting), we investigated intracellular localization of OPA3. Normal fibroblasts were electroporated with Green Fluorescent Protein tagged OPA3 fusion proteins (ex 1-2 and ex 1-3 transcripts) with or without mutated mitochondrial and/or peroxisomal targeting signals. Confocal microscopy clearly demonstrated a mitochondrial localization for both OPA3 transcripts (ex 1-2 and ex 1-3). However, only the ex 1-2 transcript also localized to peroxisomes. This rare dual localization could help elucidate the biochemical function of OPA3. To further explore OPA3 function, we created zebrafish models using antisense morpholinos. The mutant fish showed unique features, including small eyes with severely affected optic nerves, delayed development, kinked tails and spastic movements - all related to the human symptoms. These zebrafish models promise to further elucidate the biochemical function of OPA3 and assist in the development of possible therapies for this devastating disorder.

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Frequent recent inter-chromosomal sequence transfers and segmental duplications in human subtelomeres. *E.V. Linardopoulou[1,2], E.M. Williams[1], Y. Fan[3], C. Friedman[1], J.M. Young[1], B.J. Trask[1,2,4].* 1) Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Department of Bioengineering, University of Washington, Seattle, WA; 3) Department of Laboratory Medicine and Medicine, University of Washington, Seattle, WA; 4) Department of Genome Sciences, University of Washington, Seattle, WA.

Human subtelomeres form the transition zones between chromosome-specific sequence and the arrays of telomeric repeats capping each chromosomal end. These zones are composed of sequences duplicated on different sets of chromosomes and are highly polymorphic in content. We wish to understand human subtelomere organization, evolution, variation, and function, as well as to learn more about the origin and consequences of segmental duplications in general. Based on our analyses of patterns and breakpoints of subtelomeric homologies, we conclude that these patchworks were created by a series of chromosome translocations involving the very tips of chromosomes. We find that non-homologous end-joining appears to be the primary mechanism of DNA repair leading to the formation of subtelomeric duplications. Once duplications arise on different chromosomes, they undergo continued homology-based sequence transfers, which are apparent from the incongruent phylogenetic relationships of neighboring sections. Ectopic repair of subtelomeres is a potent force for recent change. Our cytogenetic analyses reveal that subtelomeric sequence moved or changed copy number at the unprecedented rate of ~0.09 times per basepair per million years during human-chimpanzee divergence. From sequence comparisons, we estimate that ~50% of known subtelomeric sequence, including ~40 new gene copies, formed through human-specific duplications or sequence transfers. Thus, subtelomeric dynamics could have both advantageous and pathological consequences in human biology. Our study suggests the existence of an evolutionary cycle between segmental polymorphisms and genome rearrangements. More generally, the translocation-based model used to explain subtelomeric duplications may be broadly applicable to other inter-chromosomal segmental duplications in the genome.

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Cloning of interstitial deletion breakpoints in monosomy 1p36. *M. Gajecka[1], C.D. Glotzbach[1], K.A. Bailey[1], L.G. Shaffer[1,2].* 1) Health Research and Education Center, Washington State University, Spokane; 2) Signature Genomic Laboratories, LLC, Spokane, WA.

Although terminal deletions of 1p36 are relatively common, interstitial deletions of 1p occur in only ~7% of cases. Little is known about the mechanism of formation of these deletions. Of the 114 monosomy 1p36 subjects ascertained to date, eight cases have one interstitial deletion and three cases have complex rearrangements with at least two interstitial deletions. Here we present the mapping and cloning of the breakpoint junctions in these subjects. To determine the sizes of the 1p36 interstitial deletions, we performed array CGH with cell lines derived from each of the three subjects using the SignatureChip^{TM} with contains BAC clones spanning the most distal 10.5 Mb of 1p36. Array CGH and metaphase FISH analyses showed that the 1p36 interstitial deletions varied in size. To identify the breakpoints, we generated somatic cell hybrids from each subject containing the derivative chromosomes 1 segregated from the normal chromosomes 1 and performed PCR and STS marker walking. After narrowing the regions containing the junctions, we used the TOPO Walker protocol (Invitrogen) to amplify across the interstitial deletion breakpoints. Junctions were identified and analysis of the DNA surrounding each breakpoint was performed. Our results indicate that the breakpoint locations, as characteristic for monosomy 1p36 syndrome, are variable in the subjects, with no common breakpoint and no common deletion size. In one subject previously identified to have two interstitial deletions, a complex rearrangement consisting of an inversion and a third, cryptic interstitial deletion, was found. Deletion breakpoints were found in unique sequences and repetitive elements in the subjects; thus, no consistent sequence motif was identified among subjects. Alignment of the deletion junctions revealed the lack of sequence similarity between the breakpoints implicating involvement of NHEJ in stabilizing these broken chromosomes.

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Delineation of chromosome 9p deletions: A model of phenotype and chromosomal mechanisms for terminal deletions. *S. Schwartz[1,2], S. Biton[2], L. Christ[2], M. Eichenmiller[2], M. Graf[3], H. Vance[4], C. Crowe[5].* 1) Univ of Chicago, Chicago, IL; 2) Case Western Reserve Univer, Cleveland, OH; 3) TGen, Phoenix, AZ; 4) Roswell Park Cancer Institute, Buffalo, NY; 5) MetroHealth Hospital, Cleveland, OH.

Over the past decade our knowledge of the phenotypic abnormalities seen in chromosomal abnormalities and mechanisms responsible for these abnormalities has increased. However, there is still little information concerning terminal deletions. We have studied 113 chromosomal abnormalities involving the deletion of a portion of chromosome 9 of which 85% of the abnormalities were de novo, and 20% of these more complex than simple deletions. Breakpoints from 65 individuals have been mapped by BAC studies and a total of 50 different breakpoints in a 9 Mb region have been identified; only in 11 breakpoints do 2 or more individuals share the same breakpoint. Breakpoints studied in greater detail have been isolated to LINE or SINE elements. A 0.5-1 Mb region has been implicated as a critical region for the characteristic facial phenotype in this syndrome; however any deletion in the 9 Mb region leads to both hypotonia and mental retardation. A deletion of only the subtelomeric region does not appear to lead to phenotypic consequences. Paternal origin of the deletions was significantly more frequent than maternal; however, there appeared to be preferential parental breakpoint regions. Results from these studies are interesting and reveal important information including that: (1) An area of increased breakage in 9p22/p23 has been identified; (2) Over 88% of the breakpoints identified are unique; most do not share the same breakpoint; (3) Although the majority of abnormalities are simple deletions a larger proportion than expected are more complex abnormalities; (4) Low-copy repeats are not implicated as a mechanism leading to these abnormalities, but other repetitive elements are important; (5) As this chromosome arm is gene poor, much of the phenotype seems similar regardless of the location of the break with the exception of the characteristic facial features; (6) The pattern of preferential regions of breakage is suggestive of a relationship with recombination differences in males and females.

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Clinical diagnosis of genomic disorders, their reciprocal products, and atypical-sized deletions and duplications by array CGH. *B.C. Ballif, C.D. Kashork, E.A. Rorem, K. Sundin, V.M. Schmitz, B.A. Bejjani, L.G. Shaffer.* Signatuare Genomic Laboratories, LLC, Spokane, WA, USA.

Genomic architectural features, such as low copy repeats (LCRs), provide recombination substrates that mediate recurrent chromosomal rearrangements that, in most instances, result in characteristic, common-sized microdeletions or microduplications. These genomic disorders alter the copy number of dosage sensitive genes resulting in genetic disease. Although the reciprocal products of these canonical microdeletions or microduplications are predicted to occur with equal frequencies, they are, nevertheless, infrequently identified. This is most likely due to the inability of conventional diagnostic methodologies to identify such rearrangements and ascertainment bias in that the reciprocal chromosomal aberrations may not be compatible with life or may produce mild, uncharacteristic, or variable phenotypes. With this in mind, the SignatureChip targeted BAC microarray was constructed with contigs of 3-6 overlapping clones for 126 clinical loci including all of the known microdeletion and microduplication syndromes. In addition, the array contains 104 control regions including contigs located 1 Mb outside of the LCRs which flank the common microdeletion and microduplication loci thus enabling the detection of typical and atypical-sized deletions and duplications. The utility of array CGH and this array format in clinical diagnostics is evident in that out of 1300 cases submitted to our laboratory we have detected the common-sized aberrations for seven genomic disorders (Sotos, Williams, AS/PWS, SMS, HNPP, NPHP1, and DGS/VCFS). Furthermore, we have identified the reciprocal products of five genomic disorders (AS/PWS, SMS, NPHP1, STS, and DGS/VCFS) and detected atypical-sized deletions or duplications in four genomic disorders (Williams, AS/PWS, SMS, and dup 17p11.2). We anticipate that array CGH will not only expand our understanding of known genomic disorders but will also facilitate the identification of variability within known syndromes by identifying reciprocal and atypical genomic rearrangements.

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Polymorphism of the palindromic sequence at the 11q23 breakpoint region affects de novo t(11;22) translocation frequency. *T. Kato[1,2], H. Inagaki[1], K. Yamada[1], H. Kogo[1], T. Ohye[1], B.S. Emanuel[3], H. Kurahashi[1,2].* 1) Div Molecular Genetics, Fujita Health Univ, Aichi, Japan; 2) Development Center for Targeted and Minimally Invasive Diagnosis and Treatment, Fujita Health Univ, Aichi, Japan; 3) Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA.

Chromosomal translocation is one of the most frequent chromosomal aberrations. Balanced carriers of a constitutional translocation usually manifest no phenotype unless an essential gene is disrupted. However, they often have problems in reproduction, such as infertility, recurrent abortion, and the birth of offspring with chromosomal imbalance. The recurrent constitutional t(11;22) is a good model for studying translocations in humans. We previously demonstrated de novo t(11;22)s in sperm samples from normal healthy males using translocation-specific PCR. In this study, we show that polymorphism of the palindromic AT-rich repeat (PATRR) identified at the t(11;22) breakpoint cluster region on chromosome 11 affects the frequency of de novo occurrence of the translocation. The typical chromosome 11 allele harbors a 450bp PATRR which comprises a nearly perfect palindrome, whereas variant alleles bear partially deleted or duplicated derivatives. Most of these variant alleles comprise asymmetric palindromes. We examined the frequency of de novo t(11;22)s in sperm from individuals with various genotypes. The typical PATRR produces de novo t(11;22)s at a frequency of ~10-5, while a short symmetrical PATRR produces them at a lower frequency. Of note, PATRRs with asymmetric centers do not produce any de novo t(11;22)s at all. We conclude that the size and symmetry of the PATRR11 are the important factors that determine the frequency of de novo t(11;22)s. It can be reasonably imagined that all symmetric PATRRs will be converted into asymmetric PATRRs in the course of human genome evolution. If this is indeed the case, our results imply that this t(11;22) translocation will rarely occur de novo in humans in the future. Palindromic regions appear to be subject to deletion to maintain genomic integrity, which is likely to protect the reproductive fitness of humans.

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Novel t(5;9)(q33;q22) fuses Itk to Syk in unspecified peripheral T-cell lymphoma. *B. Streubel[1], U. Vinatzer[2], M. Raderer[3], A. Chott[1].* 1) Department of Pathology, Medical University of Vienna, Vienna, Austria; 2) Department of Gynecology, Medical University of Vienna, Vienna, Austria; 3) Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria.

Among peripheral T-cell lymphomas the heterogenous category of unspecified peripheral T-cell lymphomas represents the most common subtype. Nevertheless, recurrent chromosomal translocations are not known to occur in this aggressive type of lymphomas. Here we describe a novel t(5;9)(q33;q22) in unspecified peripheral T-cell lymphoma. Molecular analyses delineated the breakpoints to Itk and Syk resulting in a previously undescribed activation of the Syk tyrosine kinase by Itk. Itk-Syk transcripts were detected in 5 of 30 (17%) unspecified peripheral T-cell lymphomas, but not in cases of angioimmunoblastic T-cell lymphoma (n=9) and ALK-negative anaplastic large cell lymphoma (n=7). In all 5 translocation-positive cases the breakpoints were identical fusing the N-terminal pleckstrin homology domain and proline-rich region of Itk to the tyrosine kinase domain of Syk. Three of the five t(5;9)(q33;q22)+ unspecified peripheral T-cell lymphomas shared a very similar histological pattern with predominant involvement of lymphoid follicles and the same CD3+CD5+CD4+bcl-6+CD10+ immunophenotype. These results demonstrate the presence of a recurrent t(5;9)(q33;q22) in a substantial minority of unspecified peripheral T-cell lymphomas which may represent a novel distinct subgroup of peripheral T-cell lymphomas.

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Epigenetic Regulation of Pericentromeric Heterochromatin during Mammalian Meiosis. *A. Khalil, D. Driscoll.*
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Mammalian meiosis is an elegant process that allows diploid progenitor germ cells to produce haploid gametes after proceeding through two rounds of cell divisions. The first division (MI) is unique and results in the separation of homologous chromosomes, while the second division (MII) leads to the separation of sister chromatids similar to a somatic cell division. However, the mechanisms by which meiotic cells regulate their two very different cell divisions are not well understood. We postulated a role for epigenetic chromatin modifications in regulating these processes. Therefore, we examined male germ cells with antibodies to various histone modifications. We found prior to the onset of MI that pericentromeric heterochromatic regions, which are enriched with histone H3 lysine 9 (H3-K9) dimethylation throughout meiosis, become enriched at late pachytene with H3 serine (S) 10 phosphorylation and at diplotene with H4-K5 and H4-K16 acetylation, but remain underacetylated at other sites examined. RNA polymerase II, which is clearly excluded from pericentromeric heterochromatin at pachytene, becomes exclusively associated with these regions from diplotene to MI. By contrast, pericentromeric heterochromatic regions at MII are not engaged by RNA pol II nor enriched with H3-S10 phosphorylation. Furthermore, we postulate that DNA sequences transcribed from pericentromeric heterochromatin during MI could participate in RNAi pathways as we found both DICER and RISC to be present during meiosis. These results are significant since they suggest: 1) that distinct chromatin modifications differentiate the two meiotic divisions; 2) a role for repetitive DNA sequences and RNAi in meiosis; 3) H3-K9 dimethylation is not sufficient to block RNA pol II elongation through heterochromatin; and 4) H3-S10 phosphorylation provides a binary switch to activate transcription in heterochromatin.

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Variation in meiotic recombination frequency for individual chromosomes. *R. Martin[1,2], F. Sun[1,2], M. Oliver-Bonet[1,2], T. Liehr[3], H. Stark[3], P. Turek[4], E. Ko[2], A. Rademaker[5].* 1) Medical Genetics, University of Calgary, Calgary, Canada; 2) Genetics, Alberta Children's Hospital, Calgary, Canada; 3) Institute of Human Genetics and Anthropology, Jena, Germany; 4) Urology, Obstetrics and Gynecology and Reproductive Sciences, University of California, San Francisco, USA; 5) Cancer Center, Northwestern University Medical School, Chicago, USA.

Meiotic recombination is essential for the segregation of homologous chromosomes and the formation of normal haploid gametes. This process is subject to stringent genetic control. Variation in the overall mean recombination frequencies per cell has been reported among human males. Here, we report variation in each individual chromosome among 8 normal human males (3 cancer patients with normal spermatogenesis and 5 vasectomy reversals) for the first time. An immunocytogenetic approach allowed analysis of pachytene cells by using antibodies to detect the bivalent synaptonemal complex (SCP1/SCP3), the centromere (CREST) and sites of recombination (MLH1). Individual bivalents were identified with centromere-specific multicolour FISH (cenM-FISH). The mean number of overall MLH1 foci per bivalent among donors was: chromosome 1 (3.75, range 3.33-4.11), 2 (3.40, 3.01-3.68), 3 (3.04, 2.70-3.33), 4 (2.67, 2.70-3.33), 5 (2.67, 2.42-2.98), 6 (2.50, 2.22-2.63), 7 (2.54, 2.20-2.73), 8 (2.23, 2.14-2.31), 9 (2.30, 2.10-2.41), 10 (2.39, 2.20-2.62), 11 (2.25, 2.08- 2.51), 12 (2.40, 2.18- 2.63), 13 (1.91, 1.80-2.00), 14 (1.89, 1.82-2.07), 15 (1.90, 1.83-1.97), 16 (1.96, 1.89-2.13), 17 (2.00, 1.90-2.13), 18 (1.78, 1.62-1.88), 19 (1.93, 1.80-2.03), 20 (1.84, 1.68-1.89), 21 (1.00, 0.98-1.00) and 22 (1.22, 1.07-1.88). There was significant heterogeneity in recombination frequencies across donors for chromosomes 1-7, 10-12 ($p<0.0001$, one-way ANOVA). Smaller chromosomes have 1 to 2 recombination sites and thus have less opportunity for variation since a minimum of one site is required for proper chromosome segregation. There was significant heterogeneity among males for the total mean recombination frequency ($p<0.0001$, two-factor ANOVA), but there was no significant difference between cancer patients and vasectomy patients ($p=0.68$, nested ANOVA).

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Natural variation in human meiotic recombination. *V.G. Cheung[1], D.A. Hirschmann[2], J. Burdick[1], M. Morley[1].* 1) Depts of Pediatrics & Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Combined Degree Program, School of Veterinary Medicine, Univ Pennsylvania, Philadelphia, PA.

The focus of this project is to characterize natural variation in human meiotic recombination. Using genotypes of over 6,000 SNP markers on members of the CEPH pedigrees, we examined the pattern of recombination between individuals and across the genome. In each family, we used SNP genotypes to determine the segments of the genome shared identical-by-descent between a child and his/her grandparents, and to identify the sites of recombination during paternal and maternal meiosis.

We found more than 20,000 recombination events from 619 meioses (314 maternal and 305 paternal). On average, there are 41 recombinations per female meiosis, compared to only 25 in male meiosis, a ratio of 1.7 which agrees with results from previous studies (Broman et al, 1998; Kong et al, 2002). We also found significantly different number of recombination events among women ($P < 10^{-14}$) and also among men ($P < 10^{-10}$). Previous linkage-based studies (Broman et al, 1998; Kong et al, 2002) demonstrated variation in recombination events only in female meiosis. We now provide linkage-based support for the cytological observations (Lynn et al, 2002) that recombination also varies among men.

Besides inter-individual variation, we also observed variation in recombination across the genome. We found previously known and new genomic regions with increased recombination frequency. There are genomic regions that are recombination jungles in both male and female meioses but also ones that appear to be gender specific.

In this presentation, we will describe the landscape of human meiotic recombination based on information from 43 CEPH families.

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Direct analysis of meiotic recombination in the human female. *E. Cheng[1], D. Piperna[1], R. Vallenta[2], T. Hassold[2].* 1) Dept OB/GYN, Univ Washington, Seattle, WA; 2) School Molecular Biosciences, Washington State Univ, Pullman, WA.

Until recently, almost all studies of meiotic recombination have been based on genetic linkage analyses. However, the application of immunofluorescence methodology now allows us to directly visualize meiotic exchanges; i.e., by analyzing cross-over associated proteins (e.g., MLH1) in pachytene stage spermatocytes and oocytes, we can determine the number and distribution of exchanges in individual gametes. This makes it possible to conduct studies that were previously impractical or impossible: e.g., detailed comparisons of exchanges among individuals; analyses of chromosome-specific variation in exchange distribution; studies to determine whether MLH1 foci localize to specific chromosomal sites; and direct analyses of interference. Accordingly, we recently initiated studies of human female meiosis and to date, have collected tissue from 11 fetal ovarian samples (with gestational ages ranging from 13-30 weeks). Our preliminary analyses indicate an average of 65-70 MLH1 foci per cell, consistent with expectations from CEPH genotype data. Several other expectations have also been met: in general, we observe at least one exchange on all chromosome arms (except the p arms of acrocentrics); exchanges are located more interstitially than in human male; and evidence for crossover interference is observed both in the number of exchange events per chromosome and in their relative spacing along the chromosome. However, other observations have been more surprising. Most importantly, the recombination process appears extremely sloppy when compared to our previous observations on human males and mouse males and females. Specifically, a large proportion of human oocytes have severe synaptic defects, a high frequency of bivalents have no MLH1 foci (exchanges), and non-homologous associations are common. These observations suggest that the high rate of human nondisjunction may well be set-up by abnormalities in the fetal ovary. If so, we would predict that errors of synapsis and/or recombination would be more common for chromosomes thought to be prone to nondisjunction (e.g., chromosome 16) than for others, and we are presently testing this hypothesis.

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A high-resolution survey of deletion polymorphism in the human genome. *D.F. Conrad, J.K. Pritchard.* Department of Human Genetics, The University of Chicago, Chicago, IL.

Recent work has shown that copy number polymorphism represents an important class of genetic variation in human genomes. However, previous studies have either had limited resolution to detect medium-sized polymorphisms, or have examined very few genomes. Here we report a new method that uses SNP genotype data from parent-offspring trios to identify polymorphic deletions. The method is applied to data from the CEPH and Yoruba samples from the International HapMap Project to provide the first high-resolution population surveys of deletion polymorphism. Our analysis identifies a total of 576 distinct regions that harbor deletion polymorphisms in one or more of the families. Rather strikingly, we estimate that typical individuals are hemizygous for around 25-50 deletions larger than 5 kb, totaling at least 500-700 kb of euchromatic sequence across their genomes. The detected deletions span a total of 149 known and predicted genes. However, overall the deleted regions are relatively gene-poor, consistent with the action of purifying selection against deletions. Deletion polymorphisms may well play an important role in the genetics of complex traits, however they go undetected in most current gene mapping studies. Our new method will permit the identification of deletion polymorphisms in high density SNP surveys of trio or other family data.

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A genome-wide haplotype map of 50 inbred strains of mice. *A.N. Kirby[1], C.M. Wade[1], I. Pe'er[1], E.J. Kulbokas[2], B. Blumenstiel[2], K. Lindblad-Toh[2], M. Zody[2], S. Gabriel[2], G. Liu[3], K. Jones[3], D. Hinds[4], D. Cox[4], K. Frazer[4], M.J. Daly[1,2].* 1) Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Affymetrix, Inc., Santa Clara, CA; 4) Perlegen Sciences, Inc., Mountain View, CA.

We present a haplotype map of the mouse with a density of >1 SNP per 20 kb on 50 commonly used classical and wild-derived strains of mice. The vast majority of the data is collected with a newly developed genotyping chip from Affymetrix, the accuracy of which is determined through inclusion of strains used in the original SNP discovery process.

We describe the analysis of genome-wide patterns focusing on the application of the map to the study of complex trait genetics including the acceleration of positional cloning efforts following on successful QTL identification, the integration of these variation patterns with gene expression data, and the comparison of inbred strains to putative ancestral strains to search for unlinked regions that may be not be compatible with each other in different homozygous states

We describe also progress and initial results from resequencing of 15 mouse strains being performed at Perlegen Sciences. This more complete view of variation enables deeper analyses and is compared with (and used to evaluate) the patterns observed in the haplotype map in the strains for which this data is available. The integration of these two emerging variation resources will provide a thorough catalog of variation and a better understanding for increasing the effectiveness of mapping complex phenotypes in mice.

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Common Deletions and SNPs are in Linkage Disequilibrium in the Human Genome. *K. Frazer, A. Kloek, X. Chen, M. Jen, J. Au-Young, D. Hinds.* Perlegen Sciences, Mountain View, CA.

Genomic DNA rearrangements, insertions and deletions, have recently been identified as frequent genetic differences between individuals. It is currently unknown whether these DNA rearrangements are largely rare variants (each found in a limited number of individuals) or common variants (minor allele frequency 10%) in the human population. If DNA rearrangements are common variants it is reasonable to assume that they are likely to contribute to phenotypic variation in complex traits. Since current analyses of complex human traits are primarily based on well characterized SNP data, it is important to not only determine if DNA rearrangements are common variants but whether or not they are in linkage disequilibrium (LD) with common SNPs, and thus are assessed indirectly through SNP-based association studies. To address these questions, we used a panel of 24 ethnically diverse individuals previously utilized for SNP discovery to identify over 100 human deletion polymorphisms ranging in size from 70 bp to 7 kb. We then examined these deletion polymorphisms in a second, independent set of 71 individuals used previously to construct a LD map of 1.6 million common human SNPs. Approximately 84% of the deletions identified in the discovery panel were also observed in the second larger set of individuals, suggesting that the majority are common variants. We then examined the allele frequencies of common deletions and common SNPs with similar ascertainment demonstrating that the allele frequency distributions of these two types of variants are almost identical. Further, we find that common deletions and SNPs are in strong LD with each other indicating a shared evolutionary history and suggesting that most common deletions are effectively assayed by proxy in SNP-based association studies.

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Power and phase based selection of single nucleotide polymorphisms for disease association mapping. *N.L. Saccone[1], S.F. Saccone[2], J.P. Rice[2].* 1) Department of Genetics, Division of Human Genetics, Washington University, St. Louis, MO; 2) Department of Psychiatry, Washington University, St. Louis, MO.

A thorough understanding of linkage disequilibrium (LD) patterns in the human genome is important for the design of whole-genome association studies of complex human disease. To reduce genotyping costs, it is of interest to select subsets of single nucleotide polymorphisms (SNPs) that capture the key LD information in a region. We have developed a tag SNP selection method that incorporates power calculations to identify SNPs that can best retain power to detect disease association when genotyped and used to represent untyped variants. The power calculations specify the threshold of LD strength, measured by either D' or r, used to define LD blocks from which SNPs are selected for genotyping; all markers not in blocks are added to complete the set of tag SNPs. A key, novel aspect of our SNP selection process is that it uses information about the phase of LD observed among markers to retain markers whose minor alleles are likely to be in coupling with an underlying disease allele. Coupling phase corresponds to higher power than the repulsion phase scenario; thus, this method provides improved power compared to a phase-blind approach. We have applied this method to genotype data from the International HapMap Project. Analysis of 28,937 SNPs on chromosome 7 having minor allele frequency (MAF) at least 10% was carried out with a range of LD thresholds and power requirements. With the most stringent threshold of |D'| = 1, our algorithm identified LD blocks covering 48% of the chromosome. From these blocks, phase-sensitive tag SNP selection resulted in 14,679 SNPs for a 49% reduction in numbers of SNPs required for genotyping. Relaxing the |D'| threshold to 0.8 improves reduction to 63%. Associated power calculations illustrate the advantages of this approach. We also demonstrate that these tag SNPs effectively represent underlying variants not included in the LD analysis and SNP selection, using a leave-one-out test to show that most (90%) of the untyped variants lying in blocks are in LD with a tag SNP and detectable in a screen that uses only the tags.

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An efficient comprehensive search algorithm for tagSNP selection using linkage disequilibrium criteria. *Z. Qin*[1], *S. Gopalakrishnan*[2], *G. Abecasis*[1]. 1) Center for Statistical Genetics, Department of Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Department of Electrical Engineering and Computer Science, Univ Michigan, Ann Arbor, MI.

Selecting SNP markers for genome wide association studies is an important and challenging task. The goal is to minimize the number of markers selected for genotyping in a particular platform and therefore reduce genotyping cost while simultaneously representing information provided by all other markers. Depending on the platform, it is also important to select sets that are robust against occasional genotyping failure. An array of methods has been proposed to effectively select these tag SNPs using various criteria. In this study, we devised an improved algorithm for tagSNP selection using the pairwise r2 criteria. We first break down large marker sets into many disjoint pieces, where more exhaustive searches can replace the greedy algorithm for tagSNP selection. These exhaustive searches lead to smaller tagSNP sets being generated for any given threshold. In addition, we evaluated multiple solutions that are equivalent according to the LD criteria to accommodate additional constraints such as platform specific SNP assay characteristics. We have written a computer program named FESTA (Fragmented Exhaustive Search for TAgging SNPs) based on this algorithm, and evaluated its performance using HapMap data from all Chromosomes and the ENCODE regions. We found that in densely typed regions, using a moderate pairwise r2 of 0.5 as the threshold for defining tagSNP sets, about 10% of all markers are selected as tags. At a more stringent threshold (r2>=0.8), about 20% of all markers are selected as tags. Simulation studies under various settings confirmed that our method performs well at selecting tagSNPs for association studies.

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Portability of the HapMap: Linkage Disequilibrium sharing between the continental populations and the Samoans of Polynesia. *R. Deka[1], W. Huang[2], Y. He[3], H. Wang[2], Y. Wang[3], Y. Wang[2], H. Li[3], D.E. Weeks[4], S.T. McGarvey[5], L. Jin[1,2,3].* 1) Center for Genome Information, Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Chinese National Human Genome Center at Shanghai, China; 3) Fudan University, Shanghai, China; 4) Dept of Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 5) International Health Institute, Brown University, Providence, RI.

Genome-wide association mapping has become a reality in understanding the genetic basis of common diseases. While the HapMap project would provide high-density single nucleotide polymorphism (SNP) data useful to assess the magnitude of the sharing of linkage disequilibrium (LD) among the three major continental populations, the utility of such data in association studies remain to be evaluated in other global populations, particularly in isolated populations with recent evolutionary histories. We have investigated the sharing of LD between the Samoan, a Polynesian population of relatively recent founding, and three continental HapMap populations (African, Chinese and European) based on ~8,000 SNPs covering half of the chromosome 21 with a mean density of ~1 SNP/1.9kb. Our results indicate LD extends much further in Samoans compared to the other three populations, and interestingly, we observe substantial sharing of LD between the Samoan and the Chinese. We also show that the tag SNPs identified (based on r^2) in the Chinese capture 90% of the information on genetic variation in the Samoans. Portability of genome-wide datasets among populations, particularly among those of recent shared ancestry, would tremendously impact on the utility of such data in association studies. This work is supported by the Chinese Human Genome Center at Shanghai and NIH grants DK59642 and ES06096.

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Testing tag SNP transferability across different population samples. *P.I.W. de Bakker[1,2], N. Burtt[2], R.R. Graham[1,2], J.N. Hirschhorn[2], L.C. Groop[3], C.A. Haiman[4], B.E. Henderson[4], M.J. Daly[1,2], D. Altshuler[1,2].* 1) Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Lund University, Malmo, Sweden; 4) University of Southern California, Los Angeles, CA.

Linkage disequilibrium between single-nucleotide polymorphisms (SNPs) makes it possible to increase dramatically the cost-effectiveness of association studies to test common variants for a role in disease. The International HapMap Project will very soon complete the construction of a dense SNP map across the entire human genome in 270 individuals from four ethnic groups. A major outstanding question is how tag SNPs chosen in one population (e.g. DNA samples used in the HapMap project) can capture the common genetic variation in another.

We genotyped more than 3,000 SNPs across 30 loci (in total spanning >3 Mbp) in almost 800 samples to evaluate the transferability of tag SNPs selected from the HapMap. Thus, in addition to the HapMap samples, we collected genotypes in samples from a range of ongoing disease studies: two Caucasian samples, three East Asian samples, one West African sample, two African American samples, a Latino sample, and a native Hawaiian sample. We find that tag SNPs picked from the HapMap samples appear to provide very good coverage of common (>5%) variants with only modest loss of power. These insights offer compelling experimental validation of an LD-based approach for association studies.

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Distribution of Genome-wide Linkage Disequilibrium among Multiple Populations. *H.J. Tsai[1,2], N. Battle[1,2], J. Kho[1,2], S. Choudhry[1,2], T.P. Speed[3], E.G. Burchard[1,2].* 1) University of California, San Francisco, CA; 2) Lung Biology Center, San Francisco General Hospital, SF, CA; 3) University of California, Berkeley, CA.

Genome-wide screen based on linkage disequilibrium (LD) patterns has been proposed as a plausible tool for gene mapping in complex traits. In this study, we genotyped 116,204 single nucleotide polymorphisms (SNPs) randomly distributed across the genome and examined genome-wide LD patterns in four different populations, consisting of Africans, Europeans, Native Americans and Puerto Ricans. We computed LD by using three different measures: D', r^2 and the log odds ratio, . The LD quantity, , has been proposed, but not commonly applied to measure LD (ref). We compared LD patterns obtained from these three measures. The results demonstrate that r^2 and provide better measures for capturing genome-wide LD patterns. In addition, the distributions of LD patterns across genome among four various populations show that there is significant variation in LD among populations. In a genome-wide average, Native Americans have the highest LD, Africans have the lowest LD, and Puerto Ricans, an admixed population, are intermediate. Our results provide evidence that is an excellent quantity for the measurement of LD. In addition, our results indicate the importance of investigating diverse populations for gene mapping in complex diseases. Reference: Edwards, A.W.F. (1963) J.Roy. statist. Soc. A, 126, 109-14.

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AN EVALUATION OF THE POTENTIAL OF THE HAPMAP FOR WHOLE GENOME ASSOCIATION IN A POPULATION ISOLATE. *P. Bonnen[1], I. Pe'er[2], J. Breslow[1], M. Daly[2,3], M. Stoffell[1], D. Altschuler[3,4], J. Friedman[1,5].* 1) Rockefeller Univ, New York, NY; 2) Whitehead Institute for Biomedical Research, Cambridge MA; 3) Broad Institute of Harvard and MIT, Cambridge MA; 4) Department of Genetics and Medicine, Harvard Medical School, Boston, MA; 5) Howard Hughes Medical Institute.

The availability of an emerging haplotype map of the human genome will facilitate genetic analyses by enabling whole genome association studies in advance of being able to score all of the available human SNPs. While such studies are predicted to be most powerful in isolated populations where increased linkage disequilibrium (LD) and decreased allelic diversity, the relative advantages of studying genetic isolates has not yet been formally evaluated using the HapMap data. In this report we used Affymetrix Centurion SNP arrays to compare the haplotype maps from 30 trios of the Pacific Island of Kosrae to the HapMap samples; 30 CEPH trios, 30 Yoruban trios, 88 unrelated Japanese, and 90 unrelated Han Chinese. A whole genome haplotype map was assembled from 113,240 SNPs typed in 30 unrelated Kosraen trios. On average, LD extended nearly two times (or more) farther in Kosrae compared to the other populations; 46 Kb in Kosrae vs. 28 Kb in the EUR samples and 14 Kb in the YRI samples. Haplotype diversity was also significantly reduced as assessed by counting of haplotypes over defined intervals with an average of 35 haplotypes accounting for 95% of chromosomes. In contrast 35 haplotypes accounted for only 50% of the total in the EUR population and 40% for the YRI population. We further analyzed these data to evaluate the effectiveness of the Affymetrix panel of 110,000 SNPs to detect a potentially causative allele, by hiding one SNP and evaluating its best correlate on the array. In Kosrae, one can capture 60% the SNPs in the genome with correlation of $r2 > 0.75$, reflecting practical sample size requirements, compared to an r2 cutoff of 0.25-0.55 in HapMap populations to capture the same fraction of SNPs. These data confirm that many fewer markers will be needed to conduct a whole genome association study in the Kosraen cohort (and perhaps other isolated populations).

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Chromosome-Scale Linkage Disequilibrium Properties Characterized by the Genotype Correlation. *J. Belmont[1], M. Strivens[1], S. Leal[1], A. Milosavljevic[1], R. Gibbs[1], N. Kaplan[2].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) National Institute of Environmental Health Science, Research Triangle Park, NC.

Linkage disequilibrium (LD) among closely-spaced polymorphic loci might be used to identify disease genes via indirect association. The International HapMap project has produced a database of genotypes from >1M SNP markers in 269 reference samples (Release 16). We have used the standardized composite linkage disequilibrium (cld - also called the genotype correlation) to investigate the LD patterns in the major populations of the HapMap database. We have organized the pairwise genotype correlations using spectral decomposition as a method for dimension reduction and annotated the results in the GENBOREE genome browser. About 70% of SNPs could be uniquely assigned to mutually exclusive subsets ranging in complexity from only 2 members to >50. Some SNP (~10%) exhibited LD with markers in multiple subsets (non-unique LD), while the remaining markers did not exhibit strong LD with any neighboring loci. The allele frequency distributions among markers in these groups showed that there is a strong tendency for markers with non-unique LD patterns to have higher minor allele frequencies whereas markers which are part of small subsets (2 - 6 markers) are skewed to low minor allele frequency i.e lower frequency alleles generally have fewer surrogates. Using the number of marker subsets per genomic interval as a diversity metric, we found that there are large magnitude variations in LD along chromosomes, among chromosomes, and among populations. For example, a pattern of increased long-range LD at the pericentromeric regions was observed for chromosome 3-6 and 10-13, but chromosomes 9, 14, 15, and 22 have reduced LD at the centromeres. These patterns were consistent in all HapMap populations indicating that they correspond to stable features of the chromosomes across the sample. This form of LD map has an extremely low computational overhead and provides convenient summary tools that will aid in association study design and analysis.

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The BRCA1 interacting helicase BACH1/BRIP1 is deficient in Fanconi anemia. *A.D. Auerbach[1], O. Levran[1], C. Attwooll[2], R.T. Henry[1], K.L. Milton[1], S. Barral[1], J. Ott[1], J. Petrini[2], H. Hanenberg[3], D. Schindler[4].* 1) The Rockefeller Univ., New York, NY; 2) Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Childrens Hospital, Heinrich Heine University, Duesseldorf, Germany; 4) Institute of Hum. Genetics, University of Wuerzburg, Germany.

Fanconi anemia (FA) exhibits extensive genetic heterogeneity. Seven of the FA proteins (FANCA/B/C/E/F/G/L) form a nuclear FA core complex that activates the monoubiquitination of FANCD2, while two complementation groups (FA-D1/BRCA2 and FA-J) are downstream of FANCD2. Here we identified the FA-J gene. Five patients from four families (two Hispanic and two Inuit) in the IFAR were selected for a genome-wide scan using the GeneChip Human Mapping 50K Array 240 Xba (Affymetrix Inc. Santa Clara, CA). Patients were selected because of the following criteria: a, unassigned complementation group; b, normal expression of the two isoforms of FANCD2; and c, suspected homozygosity by descent. A shared 6 Mb region of homozygosity on chromosome 17q23 was found. From this interval we chose BRIP1/BACH1 as a candidate gene based on its biological functions. Sequencing BRIP1 revealed a c.2533C>T nonsense mutation in exon 17 (R798X). Surprisingly, all five patients were homozygous for this mutation, which is predicted to create a truncated protein lacking the BRCA1-binding domain. Consistent with this, the mutated lymphoblastoid cell lines (LCLs) showed no expression of wild type BACH1 protein by WB with an antibody that recognizes the C-terminal part of BACH1. Haplotype analysis indicates that the Inuit patients share the same haplotype, while each of the Hispanic patients presents a unique haplotype. Next we screened for the c.2533C>T mutation in DNA from an additional 18 IFAR patients for whom known FA genes had been excluded. Of these, 3 more patients were homozygous and 3 were compound heterozygotes, each with a unique mutation as the second abnormal allele. Our data from functional studies of BACH1 deficient IFAR cells suggest that DNA double strand breaks are the lesions that underly the pathology of FA-J and that the FA pathway converges on BRCA1 and the BACH1 as mediators of chromosome maintenance downstream of FANCD2.

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Fanconi anemia proteins and BLM, the Bloom syndrome DNA helicase, are involved in ALT telomere maintenance. *H. Root[1], D.J. Stavropoulos[1,2], M.S. Meyn[1,2].* 1) Genetics & Genomic Biology, Hospital for Sick Children, Toronto, ON; 2) Molecular & Medical Genetics, Univ of Toronto, Toronto, ON.

Fanconi anemia and BLM proteins have been implicated in genetic recombination, a process thought to be involved in ALT (Alternative Lengthening of Telomeres) telomere maintenance. We now report data supporting interactive roles for the FA and BLM proteins in ALT.

We find that the FA proteins FANCD2, BRCA2 (FANCD1), FANCA and FANCG localize to telomeric foci in ALT human fibroblasts, but not in telomerase-positive or primary fibroblasts. Colocalization of FANCD2 and telomeres primarily occurs within PML bodies in late S/G2. Association of FANCD2 with ALT telomeres may depend on BLM, as ~90% of FANDCD2 foci that colocalize with TRF1 also co-localize with BLM. It has been proposed that ALT cells harbor dysfunctional telomeres that trigger a DNA damage response, as H2AX, a marker of double strand breaks, localizes to some ALT telomeric foci. FANCD2 is almost always present in those foci. BRCA2 localization to ALT telomeres occurs primarily in the presence of FANCD2, consistent with a role for FANCD2 in BRCA2 damage-induced foci formation.

Co-IP experiments indicate ALT-specific in vivo interactions between FANCD2, BLM, and the telomeric protein TRF2 in late S/G2. In support of a functional role for FA and BLM proteins in ALT, we can stably suppress FANCD2 expression with shRNA in telomerase positive cells but not in ALT cells. We also find that transient reductions in FANCD2 expression strongly impairs growth of ALT fibroblast cultures while transient reductions in BLM expression cause ALT-specific increases in dicentric formation and end to end fusions.

Our results indicate that the presence of FA, BLM and other recombination proteins at ALT telomeres is dynamic, with FA and BLM proteins spatially and temporally poised to assist in replication and maintenance of telomeres. Our data indicate a critical role for FANCD2 in ALT telomere maintenance and support a model in which a normal function of the FA pathway is to help cells deal with dysfunctional telomeres.

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Analysis of homology-directed DNA double-strand break repair in Fancd2-deficient mouse embryonic stem cells.
H. van de Vrugt, S. Komaki, M. Grompe. Molecular & Medical Genetics, OHSU, Portland, OR.

Fanconi anemia (FA) is a genomic instability disorder, characterized by cancer predisposition and bone marrow failure. Including FANCD2 and BRCA2, nine FA genes have been identified, the products of which operate in a genomic caretaker pathway. The FANCA,-B,-C,-E,-F,-G and -L proteins are all required for the activation of the FANCD2 protein by monoubiquitination, which occurs in S-phase and in response to DNA damage. Activated FANCD2 relocates to nuclear foci that also contain BRCA1 and BRCA2. Despite extensive characterization of the FA pathway and the associated proteins, the fundamental molecular defect in FA cells remains unknown. Previously, it has been reported that ES cells with a truncated Brca2 protein display an increase in error-prone homology-directed repair of DNA double-strand breaks (Tutt et al. 2001). Considering the convergence of the FA and BRCA genomic caretaker pathways we set out to determine whether Fancd2-deficient ES cells display a similar homology-directed DSB repair response as Brca2-truncated ES cells. Fancd2-deficient and corrected ES cells were established carrying a single copy of a direct repeat reporter construct. These cells were transfected with I-SceI to induce a DSB in a partial Bsd reporter gene. Recombination between the homologous repeats, i.e. two inactive Bsd selection markers, generates a functional selection marker and homology-directed DSB repair events were identified by selecting for blasticidin resistant ES cell clones. Our preliminary results indicate that Fancd2-deficient and corrected ES cells have similar blasticidin survival frequencies after I-SceI DSB induction, suggesting the efficiency of homology-directed repair of induced DSBs is not compromised in absence of Fancd2. Analysis of the reporter construct after DSB repair to characterize the nature of the recombination event is in progress. Moreover, the blasticidin-mediated selection of recombinant ES cell clones allows us to analyze spontaneous and DNA damage-induced recombination events within the reporter construct. Reference : Tutt, A. et al. (2001) EMBO J. 20: 4704-16.

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SNM1 acts in a BRCA1-dependent pathway non-epistatic to Fanconi anemia for genome stability. *A.W. Hemphill, Y. Akkari, J. Hejna, S. Olson, R.E. Moses.* Dept Molecular & Medical Gen, Oregon Health & Science Univ, Portland, OR.

The gene encoded by SNM1 in *Saccharomyces cerevisiae* has been shown to act specifically in the repair of DNA interstrand crosslinks (ICLs). There are five mammalian homologs of *SNM1*, including Artemis. Artemis is involved in V(D)J recombination, and mice deficient in Artemis develop severe combined immunodeficiency syndrome (SCID). Mice with a mutation in *Snm1* are sensitive to the ICL agent Mitomycin C (MMC). To explore the role of SNM1 and its homologs in ICL repair in human cells, we used siRNA to deplete SNM1, SNM1B, and Artemis in human fibroblasts, with cell survival and chromosome radials as the end points for cellular sensitivity and genome stability. Depletion of either SNM1 or SNM1B increases sensitivity to ICLs as detected by both end points, while depletion of Artemis does not. Thus SNM1 is active in maintenance of genome stability following ICL formation. To study the epistatic relationship between SNM1 and other ICL repair pathways, we used siRNAs to deplete SNM1 in a Fanconi anemia (FA) cell line. FA is a recessive disorder characterized by sensitivity to ICL agents, including MMC, manifest by increased cell death and genome instability. We find that depletion of SNM1 in an FA cell line produces additive sensitivity to MMC. We have previously shown that BRCA1 acts in the FA pathway for ICL repair, plus at least one other. Upon depletion of both SNM1 and BRCA1 in a normal cell line, we saw no increase in sensitivity above that of BRCA1 alone. We conclude that SNM1 represents a second BRCA1-dependent pathway for ICL repair, distinct from the FA pathway.

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GENOME WIDE ANALYSIS OF ALLELIC IMBALANCE IN TUMOR EPITHELIUM AND STROMAL IN PATIENTS WITH BRCA1 AND BRCA2 MUTATION POSITIVE HEREDITARY BREAST CANCER. *F. Weber[1], L. Shen[2], K. Sweet[1], K. Cooper[1], C.D. Morrison[3], T. Caldes[4], C. Eng[1].* 1) CCC,HCG,MVIMG; 2) Div of Epidemiology and Biometrics; 3) Dep of Pathology, The Ohio State University; 4) Laboratory of Molecular Oncology, San Carlos University Hospital.

Distinct molecular and clinical features different from those found in sporadic breast cancer characterize BRCA1/2 mutation carriers. The dynamic interaction between epithelial and mesenchymal cells altered by genetic alterations might promote these differences. We investigated the pattern of genomic alterations in the tumor epithelium and stroma of patients with hereditary breast cancer.51 samples were subjected to laser capture microdissection and DNA extraction from the tumor epithelium and stroma. Of these, 29 are BRCA1 and 20 are BRCA2 mutation positive. Genotyping was performed with 371 microsatellite markers using fluorescence detection method.

19375 informative markers could be evaluated for loss of heterozygosity (LOH). The proportion of LOH was 60% in the stroma and 67% in the epithelium. Hirachical cluster analysis showed a strong cluster of BRCA1 mutation positive cases. We also found for 15 out of 51 subjects the epithelium and stroma group immediately together, 10 of which are BRCA1 mutation positive. Several regions could be identified as LOH hot spot in epithelium or stroma and correlated to clinical data.The high frequency of LOH manifests the functional effect of BRCA1/2 mutations; disruption of the DNA repair mechanism. The subsequent genetic events can alter the mammary gland architecture as a determining factor for cancer susceptibility. The disproportional representation of BRCA1 mutation positive cases for which epithelium and stroma cluster directly together lend evidence for an epithelial to mesenchymal transition. Our data indicate that BRCA1/2 deleterious mutation and variants show similar high degree of genomic instability. This is also reflected in a patient with BRCA1 mutation who received prophylactic surgery. Our data underscore the interplay of tumor epithelium and its stroma in the development of hereditary breast cancer.

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Most somatic mutations in cancer occur in a novel nine-nucleotide purine-rich (NNUPURI) motif. *N. Makridakis*[1], *L. Ferraz*[1,2], *J. Reichardt*[1,3]. 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Current address: University of Campinas, São Paulo, Brazil; 3) Current address: Plunkett Chair of Molecular Biology (Medicine), University of Sydney, Sydney, Australia.

Somatic mutations are hallmarks of cancer progression. We screened 26 matched human prostate tumor and constitutional DNA samples for somatic alterations in the SRD5A2 (steroid 5?-reductase type II), HPRT (hypoxanthine-guanine phosphoribosyltransferase), and HSD3B2 (3?-hydroxysteroid dehydrogenase type II) genes, by direct sequencing of specific PCR products. We identified a total of 71 somatic substitutions in these genes. All patients analyzed had somatic alterations in at least one of these loci. Interestingly, 77.5% (55/71) of all somatic substitutions occur at a nine-nucleotide purine-rich (NNUPURI) motif with up to one nucleotide mismatch (out of nine). We also found this motif in 88.5% (23/26) of the H-ras gene somatic mutations formed in the SENCAR mouse skin model, after induction of error-prone DNA repair following mutagenic treatment. Transitions account for 92% of the H-ras somatic mutations detected in the SENCAR model and 93% of the somatic substitutions that we found in all three genes analyzed. Moreover, examination of the published literature revealed that the NNUPURI motif accounts for 66.1% (37/56) of all somatic prostate cancer mutations found in the human genes analyzed (H-ras, N-ras, Ki-ras, PTEN, p53 and the androgen receptor (AR) gene), and for 73.8% (90/122) of all somatic breast cancer mutations found in the 11 genes analyzed. The high prevalence of the NNUPURI motif in each case mentioned above cannot be explained by chance ($p < 1.32 \times 10^{-9}$). These data suggest that most somatic nucleotide substitutions in human cancer may occur in sites that conform to the NNUPURI motif. These mutations may be the result of a specific molecular mechanism, such as error-prone DNA repair.

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Loss of p21 permits carcinogenesis from chronically damaged liver and kidney epithelial cells despite unchecked apoptosis. *H. Willenbring[1], A. Vogel[1], A. Rothfuss[1], S. Eftekhari[1], M. Al-Dhalimy[1], M. Finegold[2], M. Grompe[1].* 1) Dept. of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR; 2) Dept. of Pathology, Texas Children's Hospital, Houston, TX.

In tyrosinemia type I accumulation of toxic metabolites due to fumarylacetoacetate hydrolase (Fah) deficiency results in chronic DNA damage and consequently the highest risk for hepatocellular carcinomas (HCCs) of any human disease. Here we show that *Fah*$^{-/-}$ hepatocytes exhibit a profound cell cycle arrest which causes mortality due to impaired liver regeneration despite the apoptosis resistance inherent to Fah deficiency. Using microarray analysis we found that Fah deficiency led to induction of proliferative as well as antiproliferative genes, foremost the cyclin-dependent kinase inhibitor p21. To test the role of p21 in the formation of HCCs we generated mice deficient in both Fah and p21. Remarkably, loss of p21 resulted in massive proliferation of hepatocytes and renal tubular epithelium in *Fah*$^{-/-}$ mice. This response was sufficient to compensate for cell loss due to unchecked apoptosis and hence facilitated survival. However, p21 deficiency caused HCCs, renal cysts and renal carcinomas in these animals. Thus, p21s antiproliferative function is indispensable for the suppression of carcinogenesis from chronically injured liver and renal epithelial cells and cannot be compensated for by induced apoptosis. Our results highlight the essential role of cell cycle control in both intrinsic suppression and targeted therapy of cancer.

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The human telomere maintenance proteins TRF2 and TRF1 are involved in an early DNA damage response. *P.S. Bradshaw[1,2], J.D. Stavropoulos[1,2], H. Tanaka[3], D. Gilley[3], M.S. Meyn[1,2,4].* 1) Genetics & Genomic Biology, Hospital for Sick Children, Toronto, ON; 2) Molecular & Medical Genetics, Univ of Toronto, Toronto, ON; 3) Medical & Molecular Genetics, Univ of Indiana, Indianapolis, IN; 4) Paediatrics, Univ of Toronto, Toronto, ON.

Human cells protect their genomes by using DNA damage surveillance networks that can activate DNA repair, cell cycle checkpoints and apoptosis in response to 4 double-strand breaks (DSBs)/genome. These same networks tolerate telomeres, in part because the TRF2 protein prevents recognition of telomeric ends as DSBs. We recently reported that TRF2 unexpectedly associates with induced DSBs (Nat Genet 37:193). We now present evidence that TRF2 and the telomeric protein TRF1 are involved in a novel cellular response to DSBs.

TRF1 forms transient foci with TRF2 that colocalize with laser microbeam-induced DSBs in human fibroblast nuclei. TRF1 and TRF2 are detectable at DSBs <3 seconds post-irradiation, earlier than ATM, a kinase that controls the major DSB surveillance network. Like many DNA damage response proteins, TRF2 is phosphoyrlated after DSB induction. Phospho-Thr188 TRF2 associates with induced DSBs but not undamaged telomeres. Multiple lines of evidence suggest that TRF2 and ATM functionally interact at DSB sites: the two proteins colocalize at DSBs, -ray induced phosphorylation of TRF2 is ATM-dependent, and over-expression of TRF2 impairs DSB-induced ATM autophosphoyration as well as ATM-dependent phosphorylation of H2AX and p53.

Our results provide evidence that TRF2 and TRF1 interact with DSB-containing chromatin, TRF2 competes with or attenuate ATM responses to DSBs and DSB-induced modifications to TRF2 may shift TRF2 from telomere maintenance to DSB repair. Our findings also indicate that human cells practice a strategic economy in using the same proteins in telomere maintenance and DSB repair.

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Detection of Hereditary Nonpolyposis Colorectal Cancer (HNPCC) among newly diagnosed endometrial cancer patients. *H. Hampel[1], J. Panescu[1], K. Sotamaa[1], D. Fix[1], W. Frankel[2], I. Comeras[1], P. Penzone[3], J. Lombardi[5], P. Dunn[5], D. Cohn[3], L. Copeland[3], L. Eaton[3], J. Fowler[3], G. Lewandowski[4], L. Vaccarello[4], J. Bell[5], G. Reid[5], A. de la Chapelle[1].* 1) Human Cancer Genetics Program, The Ohio State University, Columbus, OH; 2) Dept of Pathology, The Ohio State University; 3) Div of Gynecologic Oncology, The Ohio State University; 4) Gynecologic Oncology & Pelvic Surgery Assoc, Mount Carmel Health System, Columbus, OH; 5) Central Ohio Gyn Oncology, Riverside Methodist Hospital, Columbus, OH.

All newly diagnosed endometrial cancer patients in Columbus, Ohio were offered participation in a study to determine the frequency of HNPCC. All tumors underwent microsatellite instability (MSI) testing using a modified Bethesda panel of markers. Patients with MSI positive (high or low) tumors, underwent genetic testing for germline mutations in *MLH1*, *MSH2*, and *MSH6* using a variety of methods including immunohistochemical staining (IHC) for mismatch repair proteins, genomic sequencing, and multiplex ligation-dependent probe amplification. Those patients identified as having HNPCC received genetic counseling and their at-risk family members were offered counseling and predictive testing. Enrollment included 566 endometrial cancer patients. To date, 540 tumors have had MSI testing. Of these, 122 are MSI positive (22.6%; 100 MSI-high and 22 MSI-low) and 418 are MSI negative. All 122 patients with MSI positive tumors have had genetic testing and six patients have been identified with germline mutations (1 *MLH1*, 2 *MSH2* (both large deletions), 3 *MSH6*). In addition, one case with an MSI negative tumor had abnormal MSH6 IHC and was subsequently found to have a mutation in *MSH6*. IHC was consistent with the mutation result in all 7 mutation positive cases while MSI failed to identify HNPCC in 1 of 7 cases. Thus, in central Ohio 1.3% (7/540) of newly diagnosed endometrial cancer patients have been found to have HNPCC. Of the seven HNPCC patients, two meet Amsterdam criteria, two meet the Bethesda guidelines, and three do not meet any published criteria for HNPCC. Four of the patients with HNPCC were diagnosed at age 50 or later.

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Molecular analysis in patients with colorectal cancer and associated tumors in the Netherlands: MSH6 founder effects and a large proportion of MSH6 mutations conferring a phenotype which differs from classical HNPCC.

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Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common autosomal dominant susceptibility syndrome, predisposing to colorectal and endometrial cancer. Germline mutations in the mismatch repair (MMR) genes MLH1, MSH2 and, to a lesser extent, MSH6 have been shown to cause HNPCC in families fulfilling the diagnostic Amsterdam-criteria (AC). Preliminary data suggest that phenotypic expression of MSH6 gene defects may differ from classical HNPCC AC-positive expression caused by mutations in the MLH1 and MSH2 genes. We analysed 130 families, not-strictly complying with the Amsterdam criteria, for HNPCC associated mutations in the MMR genes MLH1, MSH2 and MSH6. Remarkably, in addition to families with germline pathogenic MLH1 (7/22) and MSH2 (3/22) mutations, half of the families carried a pathogenic mutation in the MSH6 gene (11/22). Moreover, two out of eight different MSH6 mutations were observed more than once in apparently unrelated families. Extended haplotype analysis subsequently demonstrated these two MSH6 mutations to be founder mutations present in the Dutch population. Clinical findings in the mutation carriers from the MSH6 positive pedigrees were in agreement with an later age at onset of HNPCC associated tumors compared to MLH1 and MSH2 mutation carriers, but a similar cumulative risk at age 65. MSH6 mutation carriers showed a lower lifetime cumulative risk for colorectal cancer compared to MLH1 and MSH2 mutation carriers. Cumulative risk for endometrial cancer was significantly higher in MSH6 mutation carriers compared to carriers of an MLH1 or MSH2 mutation and almost twice as high at age 65+. Our data suggest that MSH6 mutations are underdiagnosed and represent a separate clinical entity compared to classical AC-positive HNPCC, as expressed in most MLH1 and MSH2 families, including a later age at onset and a broader range of malignant abnormalities. Initial MSH6 gene mutation analysis is suggested in Amsterdam-negative or atypical HNPCC families with an MSH6 phenotype.

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The effect of study design on the inference of human population structure. *N.A. Rosenberg[1], S. Mahajan[2], J.K. Pritchard[3], M.W. Feldman[4].* 1) Bioinformatics Program, Univ of Michigan, Ann Arbor, MI; 2) Dept of Biological Sciences, Univ of Southern California, Los Angeles, CA; 3) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 4) Dept of Biological Sciences, Stanford Univ, Stanford, CA.

Previously (Science 298:2381-2385, 2002), we observed that without using prior information about individual sampling locations, a clustering algorithm applied to 1056 individual multilocus genotypes from 52 worldwide human populations produced genetic clusters largely coincident with major geographic regions. Reanalyzing our data, Serre and Pääbo (Genome Res 14:1679-1685, 2004) argued that the degree of clustering is diminished by use of samples with greater uniformity in geographic distribution. However, because the study design differed between their analyses and ours, it is difficult to assess the extent to which differences in outcomes arose from differences in the geographic dispersion of the samples, or from differences in sample size, number of loci, number of clusters, or assumptions about allele frequency correlations across populations. Expanding our earlier data set to 993 markers, we systematically examine the influence of these variables on the clusteredness of individuals. Among the variables considered, the number of loci and the assumptions regarding allele frequency correlation are observed to have the greatest impact. With all other variables held constant, geographic dispersion is seen to have little effect on the degree of clustering. Thus, the discrepancy from our results discussed by Serre and Pääbo is likely attributable to other factors, in particular, their use of a smaller sample size and their assumption that allele frequencies are uncorrelated across populations. As correlation coefficients 0.6 between allele frequencies on separate continents belie this assumption, our analysis of the 993 loci accounting for such correlations corroborates our earlier results: if enough markers are used with a large enough worldwide sample, individuals can be divided into genetic clusters that match major geographic subdivisions of the globe, with individuals from intermediate geographic locations having mixed membership in the clusters that correspond to neighboring regions.

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Genetic Structure of Asian Indian Populations: Genome-wide analysis of variation at 1200 DNA markers. *P.I. Patel[1, 3], S. Mahajan[2], C. Gonzales[1], L. Nino-Rosales[3], V. Ninis[3], P. Das[3], M. Hegde[4], L. Molinari[4], G. Zapata[4], J.L. Weber[5], J.W. Belmont[4], N.A. Rosenberg[6].* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Department of Biological Sciences, University of Southern California, Los Angeles, CA; 3) Department of Neurology, Baylor College of Medicine, Houston, TX; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Marshfield Clinic Research Foundation, Marshfield, WI; 6) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Although India comprises over one-sixth of the world's human population, individuals from India have generally not been included in large genomic surveys of genetic variation. We studied 672 individuals from 17 Indian populations at a collection of 1200 autosomal microsatellite and insertion/deletion polymorphisms, of which 925 overlap with markers genotyped in the HGDP-CEPH Human Genome Diversity Panel. When performing cluster analysis of individual multilocus genotypes, combining the Indian genotypes with the diversity panel data, we found that populations from India, and those from South Asia more generally, constituted one of the major human ancestry subgroups. In the combined analysis, the six main genetic clusters correspond to Africa, Europe and the Middle East, South Asia, East Asia, Oceania, and the Americas, with South Asia displacing an isolated population previously identified as the sixth cluster. Surprisingly, despite the cultural, geographic, and linguistic diversity of the 17 sampled Indian groups, genetic differentiation within India is smaller than that within comparable regional subdivisions of the human population, with $F_{st}<0.01$ across Indian populations.

Taken together, these results suggest both that many of the common genetic variants in India will not be identified without incorporating Indian groups into genome-wide investigations, but that most of these variants will be found if at least one population from India is included. Consequently, we recommend that a sample from India be incorporated alongside better-studied populations in future genomic surveys.

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European Population Structure: Ability to distinguish North and South. *M.F. Seldin[1], R. Shigeta[1], P. Villoslada[2], C. Selmi[1], L. Klareskog[3], P.K. Gregersen[4].* 1) UC Davis, Davis, CA; 2) Cent Applied Med Res, Pamplone, Spain; 3) Karolinska Univ Hosp, Stockholm, Sweden; 4) North Shore-LIJ Res Inst, Manhasset, NY.

Population structure is potentially important in clinical epidemiologic studies and in conducting association tests. This study examines population structure in a diverse group of Europeans and European Americans. After exclusion of subjects with evidence of non-European origin using ancestry informative markers, the remaining 932 subjects of nearly exclusive European ancestry were examined with a genome-wide SNP linkage typing panel. Using 2756 SNPs chosen to reduce or eliminate LD (minimum separation of 500 kb between markers), analyses were performed with both STRUCTURE and GENECLASS2 (Mountain-Rannala algorithm). The ability to distinguish populations based only on SNP typing as being derived from southern European countries or regions compared with northern European countries or regions was consistently observed and reproducible under assumptions of k (number of populations)ranging from 2 to 8 or using leave-one out cross-validation studies e.g. for k=2: 78/87 Italian and 51/78 Spanish subjects had >90% membership in the south population, where as 84/90 Swedish subjects had > 90% membership in the north population. Groups of European Americans who reported specific grandparental European origin provided additional support for this major division in European population structure: 143/162 subjects with reported Western European heritage had >90% membership in the north population. Similar results were found for Eastern Europe, whereas self identification with southeastern European origin (Italian, Spanish and Portuguese)showed predominant membership in the south population group. Finally, small groups of subjects including 1)Sephardic Jewish Americans (3/3 >90% south European); 2)Portuguese (2/3 >90% south) and 3)the only Basque subject within the Spanish collection (>85% north) provided additional support for this division. These results and identification of smaller sets of the most informative markers suggest that this major partition in European population structure may be applicable to a variety of clinical and genetic studies.

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From a still small voice - the recent evolution of a new gene VOC1 within a new locus regulating vocal development. *R.A. Clarke, C.E. de Brock, Z.M. Fang.* St George Hosp, Sch Medicine, Univ of NSW, Kogarah, Australia.

The human voice has evolved through a coordinated interplay of anatomical and neurological developments. Here we report the first voice gene (VOC1) disrupted within a family with a congenital (anatomical and neurological) vocal defect. Heterozygous individuals present with limited vocal range often reduced to a whisper. VOC1 is a new gene that has recently evolved within a new locus implicated in vocal development. The recently evolved VOC1 gene is present within all primates tested and absent from mouse and rat. VOC1 evolution is not the result of gene conversion nor does VOC1 share any significant degree of homology elsewhere within the genome. VOC1 evolved during a relatively recent rearrangement between two adjacent syntenic blocks of the genome. VOC1 mRNA transcripts are expressed across a wide range of tissue types. VOC1 represents a genuine 'new gene' that has evolved in recent evolutionary history. The new status of the VOC1 gene may prove timely in human development when consideration is given to the dependence of human speech and language on parallel vocal development. The first two speech/language genes described recently 1) FOXP2 is a transcription factor involved in a range of developmental processes in addition to the regulation of some cognitive aspects of normal speech development 2) ATP13A4 appears to be a more ubiquitously expressed cation transporting ATPase. As expected, FOXP2 and ATP13A4 have close homologues in other species including mouse suggesting their likely recruitment to speech developmental processes within the brain rather than a driving force. The very recent emergence of the VOC1 gene within a recently rearranged locus is compelling evidence for the emergence of a new vocal genotype in parallel with the evolution of human voice/speech.

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Evolutionary genetics of normal variation in human skin pigmentation. *H.L. Norton[1], E.J. Parra[2], R.A. Kittles[3], M.D. Shriver[1].* 1) Department of Anthropology,Penn State University; 2) Department of Anthropology, University of Toronto at Mississagua; 3) Human Cancer Genetics. Ohio State University.

Human pigmentation is dramatically variable among the populations of the world. Although much is known regarding the genetics of rare Mendelian hypopigmentation disorders, normal variation has been less well studied. Given the variation among world populations, two analytical methods stand out as ideally suited to study the evolutionary genetics of skin pigmentation: admixture mapping and differentiation-based screens for selection. We have surveyed several pigmentation candidate genes (TYR, OCA2, ASIP, MATP and MC1R) for signatures of selection across representative populations from six global regions: West Africa, Europe, South Asia, East Asia, Meso America, and Island Melanesia and have used admixture mapping in African-American and African-Caribbean population samples to test for admixture linkage. With the exception of MC1R, these genes all show significantly high locus-specific pairwise Fst relative to empirical distributions. These four genes also show evidence for admixture linkage in this and other studies. We next assayed the HGDP-CEPH collection of 52 populations for these SNPs. Two of these genes (OCA2 and ASIP) support a common origin for some of the variants leading to dark skin, while the other two genes (TYR and MATP) show evidence for independent origins of some of the genes underlying light skin in Europeans and East Asians.

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Non-synonymous coding SNPs from multiple populations reveal extensive gene-gene interactions in the human genome. *Z. Bochdanovits, P. Heutink.* Dept Medical Genomics, VU Univ Med Ctr, Amsterdam, Netherlands.

The existence of non-additive (epistatic) gene-gene interactions is widely accepted but how important their contribution is to phenotypic variation in humans is yet to be determined. To address this issue we performed a search for functional gene-gene interactions in the human genome. When SNP genotypes are available form multiple populations genetic differentiation at individual loci can be calculated; values that are larger than can be expected from neutral processes alone are indicative of directional selection. We extended this approach to calculate genetic distances (detect signatures of selection) at pairs of loci. Each individual possesses one out of four possible combinations of any two SNPs; i.e. is homozygous for both, heterozygous for both or homozygous for one and heterozygous for the other (2x). If selection favors one of these configurations (haplotypes) above the others, than genetic divergence at the level of haplotypes is indicative of functional epistatic interaction between the SNPs. Based on SNP genotype data from three human populations (Asian, African-American and Caucasian; dbSNP Build 124) we estimated haplotype frequencies for 12,875,275 combinations of 5075 non-synonymous coding SNPs (NSCS) using the EM-algorithm. The distribution of Fst values derived from the NSCS haplotypes were significantly different (right skewed) from the distribution based on 5075 randomly chosen neutral SNPs (NS). Because random (demographic) processes are expected to have a similar effect on all SNPs, a difference between these two classes should be the result of selection on functional interaction between NSCS. The average number of interactions per SNP was significantly larger in NSCS vs NS; the excess represents true functional gene-gene interactions. Interestingly, the magnitude of the (proportional) excess in the number of interactions, was largest at an intermediate level of genetic differentiation. Apparently, gene-gene interactions that cause a mild phenotypic change subject to intermediate levels of selection have the largest contribution to the evolved genetic divergence between human populations.

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Functional importance of evolutionarily conserved noncoding regions in humans demonstrated using HapMap data. *J. Nemesh[1,2], J.A. Drake[1,2], C. Newton-Cheh[2,3], J.N. Hirschhorn[1,2].* 1) Program in Genomics/Genetics, Children's Hospital/Harvard Medical School, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Cardiology, Massachusetts General Hospital, Boston, MA.

Evolutionarily conserved noncoding regions (ECRs) are hypothesized to be enriched for functional regulatory variants, but alternatively they could largely consist of regions with lower mutation rates during mammalian evolution. It is possible to distinguish between these alternatives: if noncoding ECRs are functionally constrained in humans, then new (derived) alleles arising within ECRs should often be subject to negative selection, and derived alleles within noncoding ECRs will be rarer than those in the remainder of the genome; by contrast, regions of low mutation rate would not alter the allele frequency distribution.

We now directly demonstrate that ECRs are functionally constrained in humans by using data from the HapMap (www.hapmap.org) to compare the derived allele frequency distributions of single nucleotide polymorphisms (SNPs) within and outside of ECRs. Specifically, we identified ECRs using the ECR browser (ecrbrowser.dcode.org), with a threshold of 80% identity with the mouse genome and 90% identity with the dog genome across 200+ bp. We compared the frequencies of derived alleles of 16,279 SNPs within ECRs and 518,705 SNPs outside of ECRs. We observed a highly statistically significant shift towards rare derived alleles for SNPs within ECRs ($p<0.0001$ by Kolmagorov-Smirnoff test; 24% vs. 22% of alleles had frequency <10%; $^2 = 72$, 1 df). We also found that the enrichment of rare alleles is unlikely to be due to ascertainment bias in HapMap, and is less dramatic in ECRs that are distant from genes. The shift towards rare alleles in ECRs is similar to that for missense vs silent variants, once ascertainment is corrected for.

We conclude that ECRs are functionally constrained in humans and are thus a potentially fertile hunting ground for disease-causing variants. Allele frequency analysis may help define classes of ECRs or parts of ECRs that are enriched for functional elements in humans.

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Genetic architecture of the extended MHC in multiple populations based on a high resolution SNP map. *M. Miretti, MHC consortium.* Wellcome Trust Sanger Institute, Hinxton-Cambridge, UK.

We constructed a genetic variation map across a 7.8Mb segment of chr 6p which harbours the extended major histocompatibility complex (MHC), in multiple populations. The 6338 SNP-based map allows to look at patterns of linkage disequilibrium (LD) which fluctuate markedly across the region. We also used the population data to estimate the recombination rates at high resolution. The distribution of the inferred recombination hotspots perfectly matched with previous sperm genotyping over the class II sub-region. Recombination rate varies across the region and is significantly lower in the the extended MHC class I sub-region containing the olfactory receptor and histone gene clusters. Between populations, recombination rates differed mainly in intensity at hotspot, which were similarly distributed. We observed multiple long-range haplotypes several of which extended over adjacent recombination hotspots. Given their population frequencies, such haplotypes can either result from population history or from selective sweeps. We are using this map to look for signatures of selection associated to ancestral HLA haplotype blocks and to select tagSNP lists representing key haplotypes. Finally, we have explored the relationship between classical HLA typing and patterns of diversity and recombination in the underlying haplotype structure. This resource will contribute to our understanding of generation and maintenance of MHC variation and aid disease association studies.

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Interparalog gene conversion pattern and evolutionary dynamics of HBII-52 C/D box snoRNAs cluster at 15q11-q12. *M. Ogorelkova[1], A. Navarro[2], X. Estivill[1].* 1) Gene & Disease Prog, Ctr Genomic Regulation, Barcelona, Catalonia, Spain; 2) Experimental and Health Sciences Department, Pompeu Fabra University, Barcelona, Catalonia, Spain.

Brain expressed, paternally imprinted C/D box HBII-52 snoRNAs are organized in a ~100 Kb cluster of 47 copies on chromosome 15q11-q12. They are suggested to post-transcritionally modify the 5HT2C transcripts by affecting the editing and/or alternative splicing. The HBII-52 copies share high homology with each other (>97%), and over half of them are expected to be functionally active. Due to high level of sequence identity, gene conversion is anticipated to be the main event shaping the DNA variability of the cluster. We have analysed the DNA variability of 25 presumably functional copies in 70 Spanish individuals. In 6.6 Kb of sequence, 34 SNPs and rare variants have been identified, 23 of which are novel. Two third of the variants have minor allele frequency less than 5%. Six variants are predicted to lead to non-functional snoRNA copies. Twenty six substitutions correspond to paralogous sequence variants (PSVs) in other copies and occur at potential gene conversion sites with tract of >5 bp. We detected 4 gene conversion events involving more than one PSV, with potential tracts between 6 and 31 bp. The overall nucleotide diversity of the cluster (4.2x10 -4) is higher than the genome average, and several snoRNA copies display very high nucleotide diversity of up to 1x10 -3. Interparalog gene conversion is expected to generate excess of sequence diversity, with excess of variants with low MAF. The spectrum of variability at HBII-52 snoRNAs cluster is strongly suggestive of frequent and recurrent gene conversion events between paralogous copies, and multiple gene conversion hot spots. Multiple SNPs are found at the proximal and distal parts of the snoRNAs cluster while central copies are devoid of variants. A recent positive selection event, possibly a fixation of human-specific copy 38, might have removed the variability within the central copies, as confirmed by tests of neutrality on preliminary data. Alternatively, some snoRNA paralogs might represent gene conversion receiver hot spots.

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Systematic sequencing analysis discloses the pattern of nucleotide polymorphisms in human microRNA genes. *A. Tajima, Y. Sakamoto, R. Mitsumori, I. Inoue.* Div Genetic Diagnosis, IMS, Univ Tokyo, Minato-ku, Tokyo, Japan.

MicroRNAs (miRNAs) are a family of small noncoding RNAs in a wide variety of organisms including humans. Recent experimental and bioinformatics studies have disclosed that over 200 distinct miRNAs are encoded in human genome. The small RNAs are enzymatically processed from hairpin-shaped precursor transcripts (pre-miRNAs), and can work as post-transcriptional repressors of target-gene expression by base-paring with the target mRNAs, implying possible associations between miRNA dysregulation and human diseases. Although miRNA biogenesis and miRNA-mediated regulation of gene expression are thought to be highly controlled in a sequence-dependent manner, there has been little attempt to characterize miRNA diversities within human populations at the nucleotide level. Here we show the genetic basis for the extent and pattern of DNA polymorphisms in human miRNA genes. We identified a total of 103 SNPs on 30,967-bp comparisons excluding indel polymorphisms by sequencing 10 selected genomic regions, which are composed of 20 distinct miRNA genes and their flanking regions, in 30 subjects (10 each from African American, European American and Japanese populations). Among the 103 polymorphic sites, three SNPs ('precursor SNPs') were observed in three pre-miRNA sequences, but none in mature ones. Within-diversity estimate for concatenated sequences of 20 pre-miRNA regions was comparable to those of the adjacent surroundings such as 5'- and 3'-flanking regions, whereas between-species comparison revealed that the 20 orthologues were highly evolutionarily conserved between humans and chimpanzees. F_{ST}-based analysis indicated that all the precursor SNPs exhibited low levels of genetic differentiation between three human populations. Considered together with additional results regarding the suppressive effect of a particular precursor SNP on *in vitro* miRNA processing and the SNP locations in hairpin structures of pre-miRNAs, the present findings suggest that purifying and/or balancing selection has shaped the pattern of nucleotide polymorphisms in human miRNA genes.

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Concordance of blastomeres in preimplantation aneuploid screening. *E. Pergament[1], C.B. Coulam[2], M. Fiddler[3], R.S. Jeyendran[4].* 1) Northwestern Reproductive Genetics, Inc; 2) Rinehart Center for Reproductive Medicine; 3) DePaul University; 4) Andrology Laboratory Services, Inc., Chicago, IL.

The testing of preimplantation embryos for aneuploidy - known as aneuploid screening - appears to be a rational yet questionable approach to enhance the initiation and outcome of pregnancies undertaken through *in vitro* fertilization (IVF). Fluorescent in situ hybridization (FISH) analyses for as few as 9 chromosomes demonstrate that the majority of human preimplantation embryos are not only aneuploid but mosaic; however, in many IVF programs transfer of embryos following aneuploid screening is based on FISH analyses of a single blastomere derived from each embryo. There have been no prospective, randomized, multicenter trials with external oversight addressing the efficacy of aneuploid screening of embryos by multicolor FISH. We report on the rates of concordance and discordance for eight chromosomes (13, 15, 16, 18, 21, 22, X and Y) between two blastomeres derived from each embryo as part of a standard IVF program in which FISH was performed by a national laboratory offering preimplantation genetic services. The study population consisted of 24 women who underwent 28 IVF cycles in which 167 of 287 (58%) embryos were successfully biopsied for two blastomeres and FISH probes were successfully applied to both blastomeres in 108 cases. In only 21% was there complete concordance for the eight chromosomes. Twenty-four percent of the embryos were discordant for one chromosome, 22% for two, 11% for three, 12% for four, 3.7% for five and six, and 1% for seven and eight chromosomes. The rate of discordance for individual chromosomes ranged from a low of 13.9% of embryos for the Y chromosome to a high of 33.3% of embryos for chromosome 15 (mean, 26.3%) There were 10 embryos (9.3%) which if transfer were based on their diploid blastomere had the potential for a trisomic conception of clinical significance. This data indicates that aneuploid screening based on FISH analyses of preimplantation embryos is unlikely to be effective in enhancing the initiation and outcome of pregnancies undertaken by IVF.

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Uniparental disomies in human preimplantation development. *A. Kuliev, S. Rechitsky, Y. Verlinsky.* Reproductive Genetics Inst, Chicago, IL.

More than half of patients undergoing IVF are 35 years or older, increasing the risk for producing gametes with age-related aneuploidies. The embryos resulting from such prezygotic errors may be predisposed to further mitotic errors, with potential of resulting in uniparental disomies through the trisomy rescue mechanism, providing one of possible explanations for imprinting disorders associated with assisted reproductive technologies (ART). To identify uniparental disomies in preimplantation embryos, we biopsied single blasomeres from the 8-cell embryos in cases of preimplantation diagnosis for age related aneuploidies, and cases of preimplantation HLA typing in patients of advanced reproductive age, using a DNA fingerprinting based on the patterns of alleles that uniquely identify an individual, relying on a multiplex fluorescent PCR of low template DNA. Using short tandem repeats (STR) with a high heterozygosity, we performed single cell DNA fingerprinting for detection of aneuploidies for chromosomes 6, 13, 16, 18, 21, 22 and X. Overall, 655 embryos were tested, showing aneuploidy prevalence of 44%, including comparable rates of trisomies, monosomies and complex errors. Uniparental disomies were detected in 15 (2.3%) cases, involving all tested chromosomes except chromosome 13. All but one uniparental disomies (uniparental disomy 16) were of maternal origin, in agreement with the predominant female meiosis origin of aneuploidies. Therefore, testing for aneuploidies in oocyte and embryos may allow not only avoiding the transfer of aneuploid embryos, but also detecting and avoiding the transfer of embryos with uniparental disomies, one of the possible contributors to imprinting disorders associated with ART.

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Spatial organization of the Prader-Willi/Angelman Syndrome region is dependent on the parent of origin. *A.D. Heggestad[1], T.R. Dennis[2], S. Rodriguez-Jato[1], B.A. Gray[2], R.T. Zori[2], D.J. Driscoll[2], T.P. Yang[1,2].* 1) Dept. Biochemistry and Molecular Biology; 2) Dept. Pediatrics, Univ. of Florida, Gainesville, FL.

The Prader-Willi and Angelman Syndrome (PWS/AS) region on chromosome 15 is composed of at least eight imprinted genes that are coordinately regulated by a bipartite imprinting center (IC). We have hypothesized that the PWS-IC acts as a positive regulator of genes expressed on the paternal chromosome via long-range intrachromosomal interactions and formation of an active chromatin hub (ACH). We now have used FISH to examine association of the PWS-IC with the distal imprinted *MKRN3*, *MAGEL2*, and *NDN* genes on the maternal and paternal chromosomes. In normal human cells, we found differential FISH patterns on the two chromosomes in which a majority of the cells showed one *MKRN3* or *MAGEL2/NDN* FISH signal that co-localized with one of the PWS-IC FISH signals, and one *MKRN3* or *MAGEL2/NDN* FISH signal that did not co-localize with the other PWS-IC FISH signal. To determine the parental origin of each FISH pattern, we used cells from AS patients with uniparental disomy (UPD) to examine the paternal chromosome, and PWS UPD cells to analyze the maternal chromosome. AS UPD cells showed preferential association of both PWS-IC FISH signals with the *MKRN3* or *MAGEL2/NDN* FISH probes, consistent with association of these distal genes with the PWS-IC on the paternal chromosome. In contrast, neither of the *MKRN3* or *MAGEL2/NDN* FISH signals associated with either of the PWS-IC FISH signals in the majority of PWS UPD cells, suggesting that these genes do not associate with the PWS-IC on the maternal chromosome. These data are consistent with a spatial organization of the AS/PWS domain in which the PWS-IC interacts with the chromosomal region containing *MKRN3*, *MAGEL2*, and *NDN* and forms an ACH specifically on the paternal chromosome. We postulate that these long-range interactions contribute to imprinting by facilitating activation of transcription of genes specifically on the paternal chromosome. We are currently investigating parent-of-origin long-range intrachromosomal interactions in this region by chromosome conformation capture.

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MLPA screening reveals subtelomeric rearrangements in holoprosencephaly. *V. David[1,2], C. Bendavid[1,2], I. Gicquel[1], M.R. Durou[2], L. Pasquier[3], S. Jaillard[4], C. Henry[4], S. Odent[3], C. Dubourg[1,2].* 1) Faculte de Medecine, Rennes1, UMR 6061 CNRS, Rennes, Cedex, France; 2) Laboratoire de Genetique Moleculaire, CHU Pontchaillou, Rennes, France; 3) Genetique Medicale, Hopital Sud, Rennes, France; 4) Laboratoire de Cytogenetique, CHU Pontchaillou, Rennes, France.

Holoprosencephaly (HPE) is the most common developmental brain anomaly in humans, usually associated with facial features. The clinical spectrum is wide, ranging from cyclopia and alobar HPE to microforms like single central median incisor. The etiology is extremely heterogeneous with environmental and genetic factors. Out of twelve redundant loci reported until 1998 in karyotype anomalies in HPE, eight were subtelomeric. Furthermore, out of the four major genes previously identified among these twelve loci, two were subtelomeric : SHH and TGIF. Therefore, we hypothesized that a subtelomeric screening in HPE patients with normal karyotypes could reveal microrearrangements in chromosomal telomeres; this could aid in refining the six remaining previously described minimal critical regions, as well as identifying new candidate loci for HPE genes. In this study we screened a cohort of 70 fetuses and 100 newborn HPE samples with normal karyotypes, even if they were already known to have mutations or microdeletions in HPE genes (SHH, TGIF, SIX3,ZIC2). We used the SALSA MLPA kit P036B human telomers from MRC Holland. Based on our results, we demonstrated that subtelomeric rearrangements, duplications or deletions, are present in HPE patients. They were associated with SHH and TGIF microdeletions in 7q and 18p respectively, but were also found in some of the other six subtelomeric loci (1q, 20p, 21q) previously described. We also pointed out that other subtelomeres defects could be involved in 1p, 5q, 8p, 9q, 16p, 17q, 18q, 22q and in 15q subcentromeric. A few cases consisted of an association between a duplication and a deletion in two chromosomal subtelomeres like 7p del and 7q dup. If confirmed, these findings will reinforce the multigenic and multihit origin usually evocated for HPE and will also offer another explanation for the wide phenotypic spectrum described in this developmental disorder.

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Novel Mutations in Human NODAL are Potentially Associated with Heterotaxy Syndrome. *B. Mohapatra[1], H. Li[1], S. Ware[3], S. Fernbach[2], T. Ho-Dawson[2], L. Molinari[2], B. Casey[4], J. Belmont[2], J. Towbin[1].* 1) Pediatrics(Cardiology), Baylor College of Medicine, Houston, TX; 2) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 3) Children's Hospital Medical center, Cincinati, OH; 4) Childrens & Womens health center, Vancouver BC V 6H 3V.

Heterotaxy is a complex congenital disorder, characterized by abnormal left-right asymmetry, involving heart and variety of visceral organs (e.g., gut, liver, lungs and spleen). The TGF- family member NODAL is known to play a critical role in vertebrate embryonic development by inducing mesoderm formation and determining left-right axis. We hypothesize that mutations in NODAL gene are associated with human heterotaxy syndrome. We have screened 300 unrelated probands with heterotaxy phenotype using PCR-based DHPLC analysis followed by DNA sequencing method and identified four missence (E203K, R275C, G260R & V284F) and a 24 base deletion with a 9 base insertion in the NODAL gene. With an exception of 1 control for G260R, all other changes are not found at least in 596 control chromosomes.All these variants except E203K are conserved across the species. Phenotype analysis of the patients carrying these heterozygous mutations revealed a high incidence of TGA, reversed heart looping and abnormal organ situs. In order to evaluate the functional roles of the identified sequence variants on the Nodal signaling pathway we have introduced these variants into the full length NODAL cDNA in pcDNA 3.1-V5-6His mammalian expression vector. A luciferase reporter assay in P19 embryonic cells by using p(AR3)-lux, p(SBE)4-lux and p(CAGA)12-lux plasmids containing down stream target promoters tagged with luciferase reporter gene demonstrated a significant inhibition of down stream target promoter activity(40-50%)with the mutants. Further analysis of Smad2 phosphorylation, an important measure of Nodal signaling, by Western blot and immunofluorescence studies using phospho-specific antibodies, also exhibited a significant reduction of Smad2/3 phosphorylation. The genotype-phenotype analysis as well as the in vitro studies, together suggests that the sequence variants observed in the NODAL are causative mutations for heterotaxy syndrome.

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Non invasive screening and rapid QF-PCR assay could reduce the need of conventional cytogenetic analyses in prenatal diagnosis. *V. Cirigliano[1], G. Voglino[2], M. Adinolfi[3].* 1) Dept Molecular Genetics, General Lab, Barcelona, Spain; 2) Molecular Genetics and Cytogenetics Lab Promea Turin, Italy; 3) The Galton Laboratory, University College London, London, U.K.

Recently it has been shown that rapid prenatal diagnosis by Quantitative Fluorescent PCR (QF-PCR) can detect the great majority of chromosome abnormalities, despite being deliberately targeted to disorders affecting three autosomes (21,18 and 13) and the sex chromosomes. Main advantages of the assay are low cost, speed and automation allowing its large scale application. We developed a QF-PCR assay that was applied on 28.000 consecutive prenatal samples with results issued in 24 hours. Most common referral indications were: raised biochemical risk (32%), advanced maternal age (30%), parental anxiety (22%) abnormal ultrasound (7 %) and increased nuchal translucency (6%). All samples were also tested by conventional cytogenetic analysis. In 26755 cases a normal chromosome complement was correctly assessed by QF-PCR without false positive results. All 1030 non mosaic aneuploidies involving chromosomes 21, 18, 13, X and Y were identified with 100% specificity. The assay also proved efficient in detecting 9/17 cases of partial trisomies and 18/36 cases of mosaicism. QF-PCR showed 100% sensitivity in detecting clinically relevant chromosome abnormalities in samples referred for advanced maternal age and increased biochemical risk. In fetuses with abnormal ultrasound the sensitivity was 95%. QF-PCR proved efficient and reliable allowing termination of affected pregnancies without waiting for cytogenetic analysis. Our results raise the possibility of reducing the load of prenatal cytogenetics if all pregnancies are also monitored by careful application of biochemical and ultrasound tests. Women with positive results are offered amniocentesis or CVS and rapid QF-PCR should be performed on all samples. In case of positive QF-PCR results further cytogenetic analyses are not necessary and, in our experience, parents have opted for early termination in all cases of autosomal trisomies. In cases of negative QF-PCR results cytogenetic analyses might only be performed for fetuses with abnormal ultrasound.

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Evidence that a deletion at the Xist locus destabilizes the DNA of the inactive X chromosome. *Y. Marahrens[1], S. Diaz-Perez[1], D. Ferguson[3], S-C. Tsai[1], Y. Ouyang[1], V. Perez[1], S. Kim[1], S. Nahas[2], R. Gatti[2].* 1) Department of Human Genetics, UCLA, Los Angeles, CA; 2) Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA; 3) Department of Pathology, University of Michigan, Ann Arbor, MI.

The inactive X chromosome of female mammals displays several properties of heterochromatin including late replication in S phase, hypoacetylation of histone H4, hypomethylation at lysine-4 of histone H3, and DNA methylation at CpG islands. Here we show that Cre-Lox mediated excision of 21-kb from both *Xist* alleles in female mouse embryonic fibroblasts led to the appearance of histone modifications normally associated with euchromatin, histone H4 acetylation and histone H3 lysine 4 methylation, throughout the inactive X chromosome. Despite the appearance of euchromatic histone modifications, the excision of 21-kb from both *Xist* alleles did not abolish Brca1 and Mecp2 localization to the inactive X chromosome and caused the inactive X chromosome to replicate even later in S phase than it does in wild type female cells. Homozygosity for the 21-kb deletion also caused the regions of the active X chromosome associated with very high concentrations of LINE-1 elements to be replicated very late in S phase. Extreme late replication is a property of fragile sites and the 21-kb deletions destabilized the DNA of both X chromosomes leading to deletions and translocations. X chromosome instability was accompanied by the phosphorylation of p53 at serine-15, a signaling event that occurs in response to DNA damage and chromatin defects. The deletions at the Xist locus also resulted in histone ?-H2AX, a histone variant that associates with double strand breaks and other DNA damage, concentrating on the inactive X chromosome. Element(s) at the *Xist* locus therefore serve to maintain the stability of the X chromosome.

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Familial and Genetic Susceptibility to Major Neonatal Morbidities in Preterm Twins. *M.J. Bizzarro[1], V. Bhandari[1], A. Shetty[1], R.A. Ehrenkranz[1], L.M. Ernst[1], B.R. Vohr[2], N. Desai[3], H.S. Bada[3], N. Hussain[4], X. Zhong[1], R. Feng[1], I. Gross[1], G.P. Page[5], H. Zhang[1], L.R. Ment[1], J.R. Gruen[1].* 1) Yale Univ, New Haven, CT; 2) Brown Univ, Providence, RI; 3) Univ of Kentucky, Lexington, KY; 4) Univ of Connecticut, Farmington, CT; 5) Univ of Alabama at Birmingham, AL.

BACKGROUND: Intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia (BPD) remain significant causes of morbidity and mortality in preterm newborns. OBJECTIVES: To assess the familial and genetic susceptibility to IVH, NEC, and BPD. METHODS: Mixed effects logistic regression and latent variable probit model analysis were used to assess the contribution of several covariates in a multicenter retrospective study of 348 twin pairs born at 32 weeks of gestation. To determine the genetic contribution, concordance rates in a subset of 160 MZ and DZ twin pairs were compared.

RESULTS: The study population had a mean gestational age (GA) of 29 weeks and birthweight (BW) of 1262 grams. Respiratory distress syndrome (RDS) ($p<0.001$), treating institution (INST) ($p<0.001$), and GA ($p<0.001$) were significant independent variables for IVH. GA ($p=0.03$) was the significant independent variable for NEC. Male gender ($p=0.01$), GA ($p=0.05$) RDS ($p<0.001$), and INST ($p<0.001$) were significant independent variables for BPD. After controlling for effects of covariates, the twin data showed that 41.1% ($p<0.001$), 37.4% ($p=0.001$), and 45.0% ($p<0.001$) of the variances in liability for IVH, NEC, and BPD respectively, could be accounted for by shared genetic and environmental factors. Amongst the 39 MZ twin pairs the observed concordance for BPD was significantly higher than the expected concordance ($p<0.001$). Without adjusting for covariates, the heritability of BPD was 48.7%. After controlling for covariates, genetic factors accounted for 58.4% ($p=0.005$) of the variance in liability for BPD.

CONCLUSIONS: Twin analyses show that IVH, NEC, and BPD are familial in origin. There is also a significant genetic susceptibility for the development of BPD in preterm newborns.

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Multilocus interactions at maternal TNF-, IL-6 and IL-6R genes predict spontaneous preterm labor in Caucasian Women. *S. Williams[1], D. Velez[1], R. Menon[2], H. Simhan[3], K. Ryckman[1], L. Jiang[1], P. Thorsen[4], I. Vogel[4], B. Jacobsson[4], S. Fortunato[2].* 1) Ctr Hum Genet Res, Vanderbilt Univ, Nashville, TN; 2) Perinatal Research Ctr, Nashville, TN; 3) Magee Women's Hosp, Pittsburgh, PA; 4) University of Aarhus, Denmark.

Preterm birth(PTB)has been shown to have a genetic component with heritability estimated to be 0.20 to 0.40. Previous studies show association of SNPs in TNF- and IL-6 with PTB. These studies have however only documented the effects of single variants or genes. Since it is probable that PTB is a multigenic phenotype, we used multifactor dimensionality reduction (MDR) to assess interactions between multiple SNPs in TNF-, IL-6 and their receptors in susceptibility to PTB. 102 Caucasian PTB mothers (<36 weeks gestation) and 325 Caucasian controls (>36 weeks gestation) were recruited. All women were between the 18 and 40 with singleton births and no preeclampsia, gestational diabetes, fetal anomalies nor placental insufficiencies. A total of 27 SNPs in the TNF-* and IL-6 pathway genes were assessed (6 in TNF-*, 6 in TNFR1, 7 in TNFR2, 5 in IL-6, 3 in IL-6R). Only 3 marginally significant allelic and genotypic associations were detected between cases and controls. Single locus analysis documented independent association of SNPs at -7294 (allele and genotype) of TNFRI and 24660 (genotype) of TNFRII loci with PTD. A multilocus model using MDR revealed a significant three site interaction between SNPs at -3448 (TNF-*), -7227 (IL-6) and 33314 (IL-6R). This interactive model predicted pheotype correctly 65.6% of the time ($p<0.001$). The high risk genotypes had an odds ratio of 3.15(95% CI 2.28-4.36, $p<0.001$). This report documents a significnat multilocus interaction that predicts PTB. Although putatively significant associations with PTD were seen at sites in TNFRI and TNFRII, they were not as predictive as the three-locus model produced by MDR, suggesting the utility of multilocus analyses in premature birth. Outcome of high-risk pregnancies may hinge on epistasis at TNF-*, IL-6 and its receptors loci. Our data suggest that interactions at these loci can be used as a screening tool for high-risk status in Caucasians.

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Complex Genetic Architecture of Birth Weight in Mexican Americans Revealed by Oligogenic Linkage Analysis.

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Birth weight is a complex phenotype with clinical and public health implications. Several studies have shown that birth weight is associated with metabolic diseases such as obesity and diabetes in adulthood. However, the genetic architecture of birth weight and its association with diseases that occur in later life is not clear. Therefore, the purpose of this study was to examine the genetics of birth weight (BW) in Mexican Americans using data from San Antonio Family Birth Weight Study subjects, all of whom had previously participated in the San Antonio Family Diabetes/Gallbladder Study, the San Antonio Family Heart Study, and the VA Genetic Epidemiology Study. For this study, both BW data and genotypic information (~10 cM map) are available for a subset of 840 individuals. Heritability of BW was 72% (p 0.0001). Using the combined map and multipoint variance components linkage analysis (SOLAR), we found the strongest evidence for linkage of BW (LOD = 3.7) at the markers D6S1053 and D6S1031 on chromosome 6q, which was replicated (LOD = 2.4) in an independent European population (Demerath et al. unpublished). Since multiple linkage peaks with some evidence for linkage (LOD 1.2) were observed on chromosomes 1q, 2q, 4q, 9p, 19p, and 19q, oligogenic models were considered that incorporated multiple loci simultaneously, and loci were added to the model sequentially until no locus met a conditional LOD score of 0.59. The joint four-locus model (LOD = 10.2) suggested that variation in BW is influenced by four QTLs: the primary QTL on chromosome 6q (D6S1053), and three others on chromosomes 2q (D2S1776), 1q (D1S518), and 9p (GATA187D09-D9S925). Some of these chromosomal regions overlap with those shown to be linked with metabolic diseases in adulthood. Thus, we found multiple loci significantly influencing variation in BW in Mexican Americans.

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A germline mutation in BLOC1S3/reduced pigmentation causes a novel variant of Hermansky Pudlak syndrome (HPS8). *N.V. Morgan[1], S. Pasha[1], J.R. Ainsworth[2], R.A.J. Eady[3], C. McKeown[4], R.C. Trembath[5], J. Wilde[6], C.A. Johnson[1], E.R. Maher[1, 4].* 1) Section of Medical & Molecular Genetics, University of Birmingham, Birmingham, United Kingdom; 2) Birmingham Midlands Eye Hospital and Birmingham Childrens Hospital, Birmingham, UK; 3) Genetic Skin Disease Group, St John's Institute of Dermatology, St Thomas' Hospital. London, UK; 4) West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham UK; 5) Division of Medical Genetics, Departments of Medicine and Genetics, University of Leicester, Leicester, UK; 6) Haemophilia Centre, Queen Elizabeth University Hospital, Edgbaston, Birmingham. UK.

Hermansky-Pudlak syndrome (HPS) is genetically heterogeneous and previously mutations in seven genes have been reported to cause HPS. Autozygosity mapping studies were undertaken in a large consanguineous family with HPS. Affected individuals displayed features of incomplete oculocutaneous albinism and platelet dysfunction. Skin biopsy demonstrated aggregates of abnormally small, but fully melanized, melanosomes. A homozygous germline frameshift mutation in BLOC1S3 (p.Gln150ArgfsX75) was identified in all affected individuals. BLOC1S3 mutations have not been reported previously in HPS patients, but BLOC1S3 encodes a subunit of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Mutations in other BLOC-1 subunits have been associated with a HPS phenotype in humans and/or mouse and a frameshift mutation in the murine orthologue of BLOC1S3 causes the reduced pigmentation (rp) model of HPS. Interestingly eye pigment formation is reported to be normal in rp but we found visual defects (iris transilluminacy, reduced visual acuity and evidence of optic pathway misrouting) in affected individuals. These findings define a novel form of human HPS (HPS8) and extend genotype-phenotype correlations in HPS.

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Loss-of-function mutations in transglutaminase 5 cause acral peeling skin syndrome. *W.H.I. McLean[1], M.A.M. van Steensel[2], E. Sprecher[3], P.M. Steijlen[2], J. van der Velden[2], S.M. Morley[1], A. Terrinoni[4], E. Candi[4], A.J. Cassidy[1].* 1) Human Genetics Unit, Univ Dundee, Dundee, United Kingdom; 2) Dermatology, Univ Maastricht, Netherlands; 3) Rambam Medical Center, Haifa, Israel; 4) Univ Rome "Tor Vergata", Rome, Italy.

Peeling skin syndrome (PSS) is an autosomal recessive disorder characterized by erythema and shedding of the outer epidermis. We studied three families where peeling was limited to the acral regions. Ultrastructural analysis revealed blister formation at the junction between the stratum granulosum and stratum corneum. By genetic linkage in an extended Dutch family, we localised the gene to a ~1 Mb region on chromosome 15q15.2, giving a significant two-point lod score of 5.7. TGM5, the gene encoding transglutaminase 5 (TG5), is located centrally in the locus. TG5 is expressed in the upper layers of the epidermis where it cross-links a variety of proteins during the cornification process. DNA sequencing revealed two homozygous missense mutations, M109T and G113C, in exon 3 of TGM5 in affected persons from the Dutch family and an unrelated Scottish kindred. Shared haplotypes suggested that this may be an ancestral mutant allele in Europe. Expression constructs were made for wild-type and mutant TG5 and were used to measure normal or mutant enzyme activity using a biochemical assay. This revealed that M109T does not significantly reduce TG5 activity and probably represents a rare polymorphism, however, G113C completely abolished TG5 activity. The atomic structure of TG5 was modelled on the published structure of TG2. This showed that the G113C mutation is located near a hinge region connecting the N-terminal domain to the active site. Perturbation of this critical region is consistent with loss of enzyme activity. In an Israeli kindred, affected persons were compound heterozygous for mutations c.255delC and c.1002+1delTG, which are predicted to result in loss of TG5 expression. This study identifies a gene for acral PSS and shows the importance of TG5 in maintaining the structural integrity of the outer differentiating layers of the epidermis.

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***CACNA1F* mutations in CSNB2 patients and characterization of a mouse model for X-linked CSNB.** *N.C. Orton[1], F.C. Mansergh[2], R. Tobias[1], T. Rosenberg[4], D.E. Rancourt[2], W.K. Stell[3], N.T. Bech-Hansen[1].* 1) Medical Genetics, University of Calgary, Calgary, Alberta, Canada; 2) Biochemistry & Molecular Biology, University of Calgary; 3) Cell Biology & Anatomy, University of Calgary; 4) National Eye Clinic, Denmark.

Mutations in *CACNA1F* cause type 2 (incomplete) congenital stationary night bliness. This variable X-linked disorder is characterized by abnormal electrophysiological response, night blindness, impaired visual acuity, myopia, nystagmus, and strabismus. DNA sequencing of *CACNA1F* in 82 patients identified 52 unique mutations (16 single base pair missense, 5 in-frame INDELS, 11 nonsense, 13 frameshift INDELS and 7 splice site). These *CACNA1F* mutations were identified among 73 patients originally diagnosed with CSNB2, Aland Eye Disease (AED), or Aland Island eye disease-like (AIED-like) suggesting that they represent the same clinical entity. The high success rate of detecting mutations in *CACNA1F* in this patient set with negative ERGs suggests that the encoded protein $Ca_v1.4$ ($_{1F}$) is important for retinal neurotransmission.

To investigate the functional effect of mutant *CACNA1F*, we created a mouse model of CSNB2 by targeted gene disruption. In characterizing this model, we observed pronounced morphological changes in second-order neurons of the adult mutant retina: ON-bipolar and horizontal cell processes extended distally from the outer plexiform layer (OPL), deep into the outer nuclear layer (ONL). Key pre-and post-synaptic components were greatly reduced in the outer plexiform layer. The dramatic reduction and disorder of photoreceptor synapses and the structural abnormalities of horizontal and bipolar cell dendrites in adult mutant *Cacna1f* mice indicate a probable role for $Ca_v1.4$ in the maintenance of photoreceptor synapses, and provides evidence for understanding the consequence of *CACNA1F* mutations in CSNB2 patients.

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Mutations in *GRM6* cause autosomal recessive congenital stationary night blindness (CSNB) with a distinctive scotopic 15 Hz flicker electroretinogram (ERG). *C. Zeitz[1], M. van Genderen[2], J. Neidhardt[1], U. Luhmann[1], F. Hoeben[2], U. Forster[1], K. Wycisk[1], G. Mátyás[1], C. Hoyng[3], F. Riemslag[2], F. Meire[2], F. Cremers[4], W. Berger[1].* 1) Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; 2) Institute for the Visually Handicapped "Bartimeus", Zeist, The Netherlands; 3) Department of Ophthalmology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

CSNB is a group of non-progressive retinal disorders characterized by impaired night vision, presumably due to a defect in signal transmission from the photoreceptor cells to the adjacent second order neurons. The mode of inheritance is autosomal dominant, recessive or X-linked. Autosomal recessive (ar) CSNB seems to be rare and so far, knowledge about the molecular basis is very limited. Here we have used a candidate gene approach and identified the underlying gene defect in families with arCSNB. Since mice lacking the metabotropic glutamate receptor 6 (*GRM6*) fail to transmit signals from the photoreceptors to ON-bipolar cells, we screened this gene for mutations in individuals of five families. Affected individuals in three of the five families carried either compound heterozygous or homozygous mutations in *GRM6*. With the standard ERG, arCSNB and X-linked CSNB1 patients could not be distinguished. Strikingly, here we show that all arCSNB patients with *GRM6* mutations displayed a distinctive abnormality of the rod pathway signals as measured with the scotopic 15 Hz flicker ERG. This novel profile suggests the existence of more than two rod pathways. The distinctive ERG feature has been observed neither in patients with X-linked CSNB1 nor CSNB patients with unknown molecular defect. Our observations will help to discriminate autosomal recessive from X-linked recessive cases by means of ERG and by molecular genetic analysis.

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SOX9 is a potent transcriptional repressor for RUNX2 during skeletogenesis. *G. Zhou[1], Q. Zheng[1], E. Munivez[2], F. Elgin[1], P. Fonseca[1], Y. Chen[2], E. Sebald[3], D. Krakow[3], B. Lee[1,2].* 1) Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Medical Genetics Institute,Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA.

Runt-domain transcription factor RUNX2 is essential for osteoblast differentiation and chondrocyte hypertrophy. However, during mouse embryonic development it is also expressed early in mesenchymal condensations. Hence, antagonism of Runx2 is required to down regulate its expression and function in those condensates that are destined to differentiate into chondrocytes instead of osteoblasts. But how this is achieved is still poorly understood. SOX9 is a transcriptional activator essential for chondrocyte condensation and cartilage formation. *SOX9* heterozygous mutations in humans cause the severe skeletal malformation syndrome campomelic dysplasia. Since *Sox9* is expressed highly in chondrogenic mesenchymal cell condensations but absent in hypertrophic chondrocytes, we hypothesize that SOX9 also acts as a transcriptional repressor for osteoblast differentiation and chondrocyte hypertrophy via inhibition of RUNX2 activity. In this study, we demonstrate that SOX9 and RUNX2 directly interact with each other through their DNA binding domains. SOX9 decreased RUNX2 binding to its target sequence and severely repressed RUNX2 transactivation of its reporter constructs. We have also generated transgenic mice expressing *SOX9* in osteoblasts by utilizing a 2.3-kb *Col1a1* promoter. The *Col1a1-SOX9*transgenic mice displayed generalized dwarfism and osteoblasts from transgenic mice were less active compared with wild-type mice. Expressions of osteoblast-related markers including *Runx2*, *Col1a1*, and *osteocalcin* were also downregulated in the long bones of transgenic mice. Furthermore, we correlated this observation in humans with campomelic dysplasia. Here, haplonisufficiency of *SOX9* resulted in upregulation of *COL10A1*, a known transcriptional target of RUNX2 during chondrocyte hypertrophy. In summary, our results suggest that an important aspect of regulating chondrogenesis is the dominant effect of SOX9 over RUNX2.

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Cranio-Lenticulo-Sutural dysplasia is caused by a SEC23A mutation resulting in abnormal COPII-mediated intracellular trafficking. *S.A. Boyadjiev Boyd[1], D. Hur[1], G. Zhang[1], W. Eyaid[2].* 1) McKusick Nathans Inst Gen Med, Baltimore, MD; 2) Department of Pediatrics, King Fahad Hospital, Riyadh, Saudi Arabia.

We described Cranio-Lenticulo-Sutural Dysplasia (CLSD, OMIM 607812) as a novel autosomal recessive dysmorphic syndrome with mild generalized skeletal dysplasia. Five males and one female in a consanguineous Saudi Arabian family have similar craniofacial features, including wide-open calvarial sutures with large and late-closing anterior fontanels, frontal bossing, hyperpigmentation of the forehead, hypertelorism, a broad and prominent nose, and Y-shaped sutural cataracts. The skeletal features also include vertebral and pelvic anomalies. A genome-wide scan detected linkage to chromosome 14q13-q21. All affected individuals were found to be homozygous for a missence mutation in SEC23A, a member of the COPII intracellular trafficking pathway. A distinct cellular phenotype of grossly dilated endoplasmic reticulum (ER) was observed in primary fibroblasts of an affected patient and unaffected carrier of CLSD. Mutations in SEDL, another member of the ER-to-Golgi trafficking pathway, have been described in patients with X-linked Spondiloepiphyseal Dysplasia Tarda (SEDT), who have a similar cellular phenotype of dilated ER. We suggest that mutations in other members of the COPII pathway may produce genetic syndromes with skeletal involvement.

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***CRTAP* is required for fibrillar collagen prolyl 3-hydroxylation and is down-regulated in osteogenesis imperfecta type VII.** *R. Morello[1], T. Bertin[1], Y. Chen[1], P. Castagnola[2], F.H. Glorieux[3], D.R. Eyre[4], B.F. Boyce[5], B. Lee[1,6].* 1) Baylor College of Medicine, Houston, TX; 2) National Institute for Cancer Research, Genoa, ITALY; 3) Shriners Hospital for Children, Montreal, CANADA; 4) University of Washington, Seattle, WA; 5) University of Rochester Medical Center, Rochester, NY; 6) Howard Hughes Medical Institute, Houston, TX.

About 10% of patients affected with Osteogenesis Imperfecta (OI) do not have type I collagen mutations or alterations, suggesting that other genes are involved in the pathogenesis of OI. The *Crtap* gene is expressed in chondrocytes, osteoblasts and osteoclasts during mouse development and after birth. To understand its function, we generated mutant mice by homologous recombination. The null mice are born viable and, although smaller, they show no obvious abnormalities at birth. However, by 3-4 months of age they develop a moderate kyphosis that becomes pronounced at 6 months of age. Radiographic analyses revealed generalized low bone mass. Histological analysis confirmed severe osteoporosis and abnormal growth plate. Bone histomorphometry demonstrated approximately 50% decrease of axial and appendicular bone volume compared to wild type mice, normal osteoblast and osteoclast numbers, but low apposition and bone formation rates. Importantly, Crtap null mice have defective osteoid formation. Moreover, because Vranka JA et. al (2004) showed that Crtap co-purifies with prolyl 3-hydroxylase, we analyzed tryptic digested, cyanogens bromide derived peptides from types I and II collagen isolated from *Crtap* null mice tissues using tandem mass spectrometry. Interestingly, the single prolyl 3-hydroxylation modification known to exist in these collagen chains was completely absent. This altered post-translation modification together with defective osteoid formation suggest a critical role of prolyl 3-hydroxylation for proper bone matrix formation. Moreover, *CRTAP* maps on 3p22, within the critical interval of OI type VII, a rare, rhyzomelic, recessive form of OI. Primary fibroblasts derived from a patient affected with OI type VII showed >10 fold decreased CRTAP mRNA and protein levels, suggesting that *CRTAP* is the gene responsible for OI type VII.

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Heterozygous mutations in *EFNA4* , the gene encoding ephrin-A4, are associated with unicoronal craniosynostosis. *E.G. Bochukova[1], A.E. Merrill[2], M. Ishii[2], D.T. Pilz[3], S.A. Wall[4], R.E. Maxson, Jr.[2], A.O.M. Wilkie[1,4].* 1) Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK; 2) USC Keck School of Medicine, Los Angeles, CA; 3) University Hospital of Wales, Cardiff, UK; 4) Craniofacial Unit, Oxford, UK.

Craniosynostosis (CRS), the premature fusion of the cranial sutures, is a common developmental anomaly affecting about 1 in 2,500 children. About 25% of CRS cases are caused by single gene defects involving *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *MSX2* or *EFNB1*, but the remainder arise from unidentified genetic and/or environmental insults. The recent discovery that heterozygous loss of function mutations in ephrin-B1 (*EFNB1*) cause X-linked craniofrontonasal syndrome in females, but that hemizygous males exhibit a paradoxically milder phenotype, suggested functional redundancy in the ephrin-Eph receptor signalling pathway. The observation of an altered protein distribution of two further members of the ephrin family, ephrin-A2 and -A4, in the defective coronal sutures of *Twist1*$^{+/-}$ mutant mice, prompted us to investigate the contribution of mutations in the human orthologues to craniosynostosis. We found no mutations in *EFNA2*, but identified three different heterozygous mutations in *EFNA4* in a panel of 81 individuals with non-syndromic coronal synostosis of unknown cause. By contrast, no *EFNA4* alterations were identified in 151 patients with other forms of CRS, or in 370 controls. Two *EFNA4* mutations (encoding H60Y and P117T) are predicted to affect ligand-receptor interaction, the third (471_472delCCinsA) introduces a frameshift into an alternative spliceform. Further analysis of the *Twist1*$^{+/-}$ mice indicates a role for ephrins in specifying the boundary between neural crest and cephalic mesoderm. Our findings identify a new genetic predisposition to nonsyndromic CRS and confirm a key role for ephrin-Eph signalling in the developing coronal suture.

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Zebrafish dax1 is required for development of the interrenal organ, the teleost adrenal cortical equivalent. *Y. Zhao[1], Z. Yang[2], J.K. Phelan[3], D.A. Wheeler[4], S. Lin[2], E.R.B. McCabe[1,3].* 1) Human Genetics, UCLA Geffen Sch of Med; 2) Molecular, Cell and Developmental Biology,UCLA; 3) Pediatrics, UCLA Geffen Sch of Med; 4) Molecular and Human Genetics, Baylor Coll of Med, Hoston, TX.

Mutations in human *DAX1*, an unusual orphan nuclear receptor, cause X-linked adrenal hypoplasia congenita (AHC). *DAX1* is expressed throughout the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis in mammals. Previously we reported the cloning of a DAX1 homologue in the zebrafish embryo. Like other non-mammalian DAX1s, the putative zebrafish dax1 contained the conserved C-terminal ligand-binding-like motif; but the N-terminal region lacked the unusual repeat motif of the DNA-binding-like domain in mammals. Whole mount *in situ* hybridization (ISH) revealed the expression of this gene in the hypothalamus, the interrenal organ and a novel bilateral symmetrical structure in the trunk during early embryogenesis. To define further the expression profile of the zebrafish *dax1*, we performed two-color ISH of *dax1* and markers for the fin bud, otic vesicle and kidney, respectively. The novel bilateral *dax1*-expressing structure was located immediately above the yolk sac, between the otic vesicle and the pectoral fin buds. We also examined the expression of *dax1* at later developmental stages. Interestingly, this gene was expressed in the liver after 3 days post fertilization (dpf) in the zebrafish larvae. To investigate the potential role of dax1 in zebrafish interrenal gland development, we used antisense *dax1* morpholino oligonucleotides (MO) to knockdown dax1 expression during early embryogenesis. Injection of *dax1* MO led to severe phenotypes similar to *ff1b* (SF1) MO injected embryos consistent with disturbance of salt metabolism, a normal function of the interrenal organ. The disruption of dax1 function was also shown to downregulate expression of steroidogenic proteins, *cyp11a* and *star*. Based on the conserved gene structure, significant protein homology, shared expression profile, and striking MO phenotypes, we propose that dax1 is the ortholog to mammalian DAX1, and is required for the normal development and function of the interrenal organ in zebrafish.

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Disruption of the microRNA pathway by the targeted loss of Eif2c2 results in aberrant primitive streak formation.*Eif2c2*. *R.S. Alisch, M. Nakamoto, T. Caspary, P. Jin, S.T. Warren.* Department Human Genetics, Emory University School of Medicine, Atlanta, GA.

MicroRNAs are a class of noncoding RNAs that play critical roles in a diverse collection of physiological and developmental processes. MicroRNAs regulate the expression of a wide variety of genes at both the transcriptional and post-transcriptional levels. MicroRNA function requires the formation of an RNA-induced silencing complex (RISC). The Argonaute family of proteins are highly conserved members of the RISC. One family member, AGO1, and its mammalian ortholog, EIF2C2, have recently been shown to interact with the fragile X mental retardation protein, whose deficiency causes fragile X syndrome. To more fully understand the function of the microRNA pathway in mammals, we disrupted the Eif2c2 locus in mice. Homozygous embryos were recovered at e7.5, but not at e8.5, in appropriate genetic ratios, indicating the loss of EIF2C2 function results in developmental arrest during gastrulation. As an initial characterization of this phenotype, we monitored the expression pattern of a primitive streak marker, Brachyury (T), in e7.5 embryos. In Eif2c2-/- embryos, T expression was expanded compared to wild-type, suggesting the microRNA pathway regulates primitive streak formation. Using gene chip microarray expression analysis on Eif2c2-/- embryos, we discovered sex-specific expression patterns for 137 genes. Because e7.5 is prior to gonadal differentiation in mice, these data suggest the microRNA pathway also may play a role in regulating chromosomal sex-specific gene expression. Interestingly, a sex-specific quantitative trait locus that modifies T expression was mapped within 2 centimorgans of Eif2c2. Together, these data suggest that EIF2C2 regulates fundamental processes during mammalian development by modifying T expression in a sex-dependent manner.

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Dissecting the genetic basis of gene expression variation in the human genome. *B.E. Stranger[1], M.S. Forrest[1], S. Tavaré[2], A.G. Clark[3], S. Deutsch[4], R. Lyle[4], S. Antonarakis[4], P. Deloukas[1], E.T. Dermitzakis[1].* 1) Wellcome Trust Sanger Institute, Hinxton, UK; 2) Centre for Mathematical Sciences, University of Cambridge, Cambridge, UK; 3) Department of Molecular Biology and Genetics, Cornell University, NY; 4) Department of Genetic Medicine, University of Geneva Medical School, Geneva, Switzerland.

The goal of this work is to identify and characterize functionally variable genomic regions that are likely to contribute to complex phenotypes and disorders in human populations through effects on regulation of gene expression. Gene expression level was quantified for ~700 genes (ENCODE, Hsa21, 10Mb region of Hsa20) in a sample of lymphoblastoid cell lines derived from 60 unrelated CEPH individuals, using Illumina Expression arrays. We performed association analyses using mRNA levels and SNP genotypes, focusing on the top 50% of genes exhibiting the most variability. We used SNPs from the publicly-available 5kb whole genome HapMap, the denser HapMap SNPs in 10 ENCODE regions, and a similarly dense SNP map of 20q12-13.2 from the Sanger Institute. We identified loci exhibiting highly significant associations between mRNA level and SNPs located *cis*- to the coding locus, including loci with associated SNPs spanning the coding locus, SNPs located 5- or 3- to the locus, and a locus with associated SNPs in the first intron. We observed genes where a *cis*- signal was detected, but its effect was spread over many SNPs and thus did not meet our significance threshold. By working with the genomic set of HapMap SNP genotypes, we were also able to identify significant *trans*-acting SNPs. This has allowed us to compare the relative frequency of *cis*- vs. *trans*-acting functional variants for these genes. For SNPs and regions showing association with expression variation, we are currently correlating known functional elements within the ENCODE regions. As the Phase II HapMap is becoming available shortly (4.5 million SNPs), by the time of the meeting we will have expanded the association analyses using this much higher resolution map. In addition, we are extending this work to include the three additional genotyped HapMap populations.

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Severely incapacitating mutations in patients with extreme short stature identify RNA processing endoribonuclease RMRP as an essential cell growth regulator. *C.T. Thiel[1], D. Horn[2], B. Zabel[3], A.B. Ekici[1], K. Salinas[4], E. Gebhart[1], F. Rüschendorf[5], H. Sticht[6], J. Spranger[3,7], D. Müller[8], C. Zweier[1], M.E. Schmitt[4], A. Reis[1], A. Rauch[1].* 1) Institute of Human Genetics, University of Erlangen, Germany; 2) Institute of Medical Genetics, Virchow-Klinikum, Humboldt University, Berlin, Germany; 3) Childrens Hospital, University of Mainz, Germany; 4) Department of Biochemistry & Molecular Biology, SUNY Upstate Medical University, Syracuse, NY; 5) Gene Mapping Centre, Max-Delbrück-Centre, Berlin, Germany; 6) Institute of Biochemistry, Department of Bioinformatics, University of Erlangen-Nürnberg, Germany; 7) Greenwood Genetic Center, Greenwood, SC; 8) Institute of Medical Genetics, Klinikum Chemnitz, Germany.

The growth of an individual is deeply influenced by regulation of cell growth and cell division, which also contribute to a wide variety of pathological conditions, including cancer, diabetes, and inflammation. In order to identify a major regulator of human growth we performed positional cloning in an autosomal recessive type of profound short stature, Anauxetic (to let not grow) dysplasia. Homozygosity mapping led to the identification of novel mutations in the RMRP gene, which was previously known to cause two milder types of short stature with susceptibility to cancer, cartilage hair hypoplasia and metaphyseal dysplasia without hypotrichosis. We show that different RMRP gene mutations lead to decreased cell growth by impairing ribosomal assembly and altering cyclin dependent cell cycle regulation. Clinical heterogeneity is explained by a correlation between the level and type of functional impairment in vitro and severity of short stature or presence of cancer predisposition. While the CHH founder mutation affects both pathways intermediately, Anauxetic dysplasia mutations do not affect B-cyclin mRNA levels but severely incapacitate ribosomal assembly via defective endonucleolytic cleavage. Anauxetic dysplasia mutations thus lead to poor processing of rRNAs while allowing normal mRNA processing and therefore genetically separate the different functions of RNase MRP.

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Identification of miRNA on chromosome 21 and their potential targets. *C. Borel[1], C. Tapparel[1], E.T. Dermitzakis[2], A. Kouranov[3], A.G. Hatzigeorgiou[3], S.E. Antonarakis[1].* 1) University of Geneva Medical School, CH; 2) The Wellcome Trust Sanger Institute, Hinxton, UK; 3) School of Medicine, University of Pennsylvania, Philadelphia, USA.

The 21 to 22 nucleotides long microRNA (miRNA) are an abundant class of non-coding RNA, that interact with mRNAs to trigger depending on the degree of complementarity with their targets either translation repression or RNA cleavage. Hundreds of miRNA genes have been reported in diverse animals, and many of these were shown to be phylogenetically conserved. To identify novel miRNA, we aligned the HSA21 genomic sequence with the syntenic mouse sequences and selected sequences between 36 and 99 bp long conserved with more than 70% identity and no gap. Note that more stringent criteria, i.e. sequences longer than 100bp, more than 70% identity and no gap were previously shown to be unsuitable to identify miRNAs, but perfect for Conserved Non-Genic sequences and coding-exons. We isolated a total of 2796 of these sequences and kept the seven candidates with the following features that characterize miRNAs: (i) folding energies below -32.5 kcal/mol; (ii) 1 to 4 nt bulges and mismatches; (iii) GC content between 32.8% and 62.5%; and (iv) evolutionary conserved secondary structures. To experimentally verify that these sequences were encoding bona fide miRNAs we tested their expression by RNase Protection Assay in total RNA extracted from 3 different cell lines. This procedure allowed to readily show that at least three of the candidates sequences hybridized to ~21-24 nt RNA. Further we analyzed the function of miRNAs on chromosome 21 with the DIANA-microT program (http://www.diana.pcbi.upenn.edu). We searched 18,000 UTRs of human genes and found more than 8 predicted target genes per miRNA. We developed a new method to screen in vitro these predictions. By trapping target genes with biotin-miRNA oligonucleotides, we identified at least 7 novel targets for the mir-let7c and mir-155. Our study provides novel insights into the identification of human miRNAs and we are currently investigating their post-translational down-regulation in order to validate their functionality in a biological context.

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CTCF binding regulates ataxin-7 gene expression: a novel system for transcription control. *R.T. Libby[1], B.L. Sopher[1], D. Cho[2], S. Baccam[1], S.J. Tapscott[2], G.N. Filippova[2], A.R. La Spada[1].* 1) Lab Medicine, Univ Washington, Seattle, WA; 2) Fred Hutch Cancer Res Ctr, Seattle, WA.

Spinocerebellar ataxia type 7 (SCA7) is a polyglutamine disease characterized by retinal and cerebellar degeneration. The ataxin-7 gene consists of 13 exons with the start site of translation and CAG repeat expansion located in exon 3. The ataxin-7 CAG repeat is flanked by two binding sites for CTCF, a transcription factor with a variety of regulatory functions including the creation of insulator and silencing domains. After we subcloned a 13.5 kb genomic fragment containing the 3 region of intron 2, exon 3, intron 3, exon 4, and the 5 region of intron 4, we inserted a 92 CAG repeat expansion to create an ataxin-7 mini-gene construct (RL-SCA7 92R-wt). To test the hypothesis that CTCF binding is a determinant of ataxin-7 expression or function, we mutated the CTCF binding site 3' to the SCA7 CAG repeat in this 13.5 kb ataxin-7 mini-gene to derive a RL-SCA7-92R-CTCF(mut) construct. After demonstrating that the RL-SCA7-92R-CTCF(mut) version had lost its ability to bind CTCF, we generated RL-SCA7-92R-wt and RL-SCA7-92R-CTCF(mut) transgenic mice. While multiple lines of RL-SCA7-92R-wt mice are normal and express negligible amounts of ataxin-7 mini-gene mRNA and protein, independent lines of RL-SCA7-92R-CTCF(mut) mice develop a phenotype of progressive ataxia and retinal degeneration. The RL-SCA7-92R-CTCF(mut) mice express ataxin-7 mini-gene mRNA and protein, with pronounced aggregate formation in cerebellum and retina. Bioinformatics analysis of intron 2 revealed the presence of an alternative promoter and the existence of a putative exon that we have designated as exon 2A. As mutation of the CTCF binding site permitted this alternative promoter to function, our results indicate that CTCF levels can modulate expression of ataxin-7. Furthermore, recent studies have established that ataxin-7 is a transcription co-factor in the STAGA co-activator complex and that impairment of ataxin-7 normal function contributes to retinal degeneration in SCA7. Our findings therefore suggest that CTCF may regulate STAGA co-activator function through its modulation of ataxin-7 expression.

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Extensive Short-Range Copy-Number Polymorphism In The Human Genome And Its Likely Affect Upon Common Disease Phenotypes. *A.J. Brookes.* Department of Genetics, University of Leicester, UK.

Our unpublished data suggests that previously unrecognized short-range Copy-Number Polymorphism (CNP) may span a substantial fraction of the human genome, and these variants may play a direct role in common disease etiology.

DNA regions of >1kb are sometimes represented multiple times in the human genome, and these are termed segmental duplications. These duplicons make up ~5% of the human genome and, as our group first reported in 2004, they almost always show copy-number variability within populations. Others have shown that a large number of long regions (>0.1Mb), enriched for known duplicon domains, also show copy-number variability between individuals. Thus, from mid-2004, it became clear that long-range CNP affects 5-10% the human genome.

We have now uncovered evidence that copy number variations; i) are often present as short (few-kb or sub-kb) domains in regions believed to be single copy, affecting 10-20% of the genome (alleles >1% frequency), ii) contain many sites erroneously listed as SNPs in public databases (and which may behave as such when genotyped on standard platforms) but which are in fact single base differences in paralogous CNP versions (we call these Multi-Site variations: MSVs), iii) include markers represented in the HapMap as normal SNPs along with allele frequency data in various populations, and iv) associate with disease state in case-control analyses, and so may have a direct effect upon gene function and disease causation.

These findings, in conjunction with emerging data on deletion and inversion polymorphism, are of critical importance for genome sequencing, population genetics, and genetic disease studies - all of which previously assumed that the human genome is far less jumbled than it actually is. This calls for new methods for genome characterization, and we are working on this by using the above observations (particularly the disease scenarios) as a test-bed for our innovations.

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The implication of the repetitive element Alu in the genetic etiology of HNPCC. *L. Li[1], S. McVety[1,3], R. Younan[2, 4], D. DuSart[5], P.H. Gordon[2], P. Hutter[6], F.B. Hogervorst[7], P. Liang[8], G. Chong[1, 3, 4], W.D. Foulkes[1, 3, 4].* 1) Human Genetics, Lady Davis Inst., Montreal, QC, Canada; 2) Dept Surgery, McGill University, Montreal, QC, Canada; 3) Dept Human Genetics, McGill University, Montreal, QC, Canada; 4) Dept Diagnostic Medicine, SMBD-Jewish General Hospital, Montreal, QC, Canada; 5) Murdoch Childrens Research Institute, Royal Childrens Hospital, Victoria, Australia; 6) Institut Central des Hopitaux Valaisans, Sion, Switzerland; 7) Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, Netherlands; 8) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, New York.

A high frequency of germ-line genomic rearrangements in *MSH2* and *MLH1*, among HNPCC patients from different ethnic populations, has been observed in the past few years. A detailed analysis of the known DNA breakpoints in the two genes was performed to explore the underlying mechanism causing this type of mutagenesis. Distinct deletion patterns were observed at the two loci, Alu mediated homologous recombinations account for 85% of the deletions in *MSH2*, but only 25% for *MLH1* ($p<0.0001$). It is notable that the average Alu density for the intronic regions of *MSH2* is 34.2% or 1.325 elements per kilobase (kb), is significantly higher than that of *MLH1*, 21%, or 0.748 elements/kb ($p<0.0001$). Both are higher than the average density of the genome, which is 13%, or 0.33 elements/kb. Plotting the distribution of the known DNA breakpoints among the introns of the two genes showed that the highest breakpoint density is co-localized with the highest Alu density. Our study suggests that, instead of being involved passively, Alu is a promoting factor for the genomic deletions in both *MLH1* and *MSH2*. This may be achieved by modulating the local organization of the chromatin, consequently contributing to its susceptibility to genomic rearrangement. At a locus predisposed to recombination, the local Alu density predicts the deletion pattern by serving as a favorable substrate for the recombination event.

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Quantitative phenotypic variation in long- and middle-wave cone photoreceptors due to polymorphism in an upstream insulator element. *J. Stamatoyannopoulos[1], R. Thurman[2], W. Noble[3], T. Kutyavin[1], A. Shafer[1], M. Dorschner[1], S. Deeb[2].* 1) Dept. of Molecular Biology, Regulome, Seattle, WA; 2) Div. of Medical Genetics, University of Washington, Seattle, WA; 3) Dept. of Genome Sciences, University of Washington, Seattle, WA.

Little is currently known about the genetic mechanisms that mediate quantitatively varying phenotypic traits. The ratio of long- (L) and middle-wave (M) cone photoreceptors in the human retina is a well-described model trait that exhibits high variability (~13-fold) between individuals. Previously, we used quantitative chromatin profiling (Nature Meth. 1:219-25, 2004) to map cis-regulatory elements in the long-wave photoreceptor (OPN1LW) locus, and resequenced these elements in males with known L:M ratios. This study revealed that inter-individual variability in the L:M ratio is regulated in cis, but not by variation in the locus control region (LCR) or OPN1LW promoter. Rather, common variation in a DNaseI hypersensitive site 7.4kb upstream of the OPN1LW gene accounts for the majority of interindividual variability in L:M ratio ($P<0.0074$). We found this novel element to be constitutively active, as revealed by chromatin profiles in retinal, neural, intestinal, lymphoid, hepatic, and erythroid cells. Here we show that variants associated with high L:M ratios disrupt a strongly evolutionarily constrained nucleotide within the core binding site for the insulator protein CTCF. This element appears to demarcate the functional boundary of the X-linked photoreceptor domain from the upstream MECP2 gene, and is conserved in all species which display conserved synteny between OPN1LW and MECP2. The results provide a novel genetic mechanism underlying common quantitative phenotypic variation.

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RSK2 mediated chromosomal remodeling of Utrophin-A promoter. *U. Basu[1], M. Gyrd-Hansen[2], T.O.B. Krag[1, 2], C.J. Jensen[2], M. Frödin[2], T.S. Khurana[1].* 1) Department of Physiology & Pennsylvania Muscle Institute, University of Pennsylvania Medical School, Philadelphia, USA; 2) Department of Clinical Biochemistry and Experimental Medicine, Glostrup Hospital, Denmark.

Duchenne muscular dystrophy (DMD) is a fatal genetic disorder caused by absence of dystrophin. Utrophin, the autosomal homolog of dystrophin can compensate dystrophins absence when overexpressed. In skeletal muscle, utrophin is synaptically enriched, in part due to the selective transcription of utrophin at sub-synaptic nuclei. Currently, utrophin transcription is known to be driven by two promoters (A and B). Although some of the molecular regulatory mechanisms have been identified, transcriptional regulation of the utrophin-A promoter is not yet fully understood. The neurite-associated growth factor heregulin activates the promoter via ERK pathway-dependant phosphorylation of the ets-related transcription factor complex GABP, which recognizes and binds the N-box motif in the utrophin promoter. However, little is known about changes at the level of chromatin that ultimately allow increased transcription of utrophin in muscle. Here, we demonstrate that heregulin phosphorylates and activates ribosomal S6-kinase 2 (RSK2) in an ERK-dependent manner. RSK2 overexpression was sufficient to increase de novo transcription from the utrophin promoter. Heregulin stimulation phosphorylates histone H3 (Ser10) in an ERK-dependent manner and increases phosphohistone H3 content of the stimulated utrophin-A promoter. Since Histone 3 phosphorylation at Ser10 is an important nucleosome structure-altering modification, RSK2 mediated remodeling of the utrophin promoter may play a critical role in transcriptional regulation of utrophin expression. These findings help further define transcriptional control mechanisms, as well as identify chromosomal remodeling of the utrophin promoter as a potential target for DMD therapy.

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Identification of a candidate FMRP phosphatase and investigation of phosphorylation as a rgulator of FMRP suppression of translation. *U. Narayanan[1], S. Ceman[2], S.T. Warren[1].* 1) Human Genetics, Emory University, Atlanta, GA; 2) Cell and Structural Biology, University of Illinois, Urbana, IL.

Fragile X syndrome is a common form of inherited mental retardation and is caused by the functional loss of the fragile X mental retardation protein (FMRP). FMRP is a RNA-binding protein that negatively regulates translation of specific mRNA ligands. FMRP has been identified as a phosphoprotein that specifically associates with stalled ribosomes when phosphorylated at serine 499. This suggests that FMRP phosphorylation may suppress translation of ligand mRNAs while dephosphorylation may be the signal to release translational suppression. Using pharmacological phosphatase inhibitors we sought to identify the FMRP phosphatase(s). Persistence of FMRP phosphorylation in cultured mouse cells in the presence of 0.5 nM okadaic acid revealed protein phosphatase 2A (PP2A) as an FMRP phosphatase. Co-immunoprecipitation analyses confirmed an association between the catalytic subunit of PP2A and FMRP in vivo. Subsequently, metabolic labeling demonstrated that FMRP remained phosphorylated in the presence of a dominant negative mutant of PP2Ac (L199P) relative to wildtype PP2Ac. These data suggest that phosphorylated FMRP may hold certain mRNAs translationally repressed on stalled ribosomes until a signal mediated by PP2A dephosphorylates FMRP resulting in the synthesis of the required protein(s) encoded by the bound mRNAs. However, it remains unclear how phosphorylated FMRP represses translation. Recently, FMRP has been known to interact both biochemically and genetically with the microRNA pathway. MicroRNAs have been shown to negatively regulate translation of partially complementary mRNAs, indeed by ribosome stalling. We show phosphorylated FMRP is preferentially associated with micoRNAs relative to unphosphorylated FMRP. Thus, the phosphorylation status of FMRP may influence microRNA association thereby regulating the translation of key messages in the brain.

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DNA methyltransferase (DNMT) variants associated with aberrant MLH1 methylation in endometrial cancer. *H. Chen[1], A. Liu[1], N. Taylor[2], D.G. Mutch[1], S. Feng[3], P.J. Goodfellow[1, 2].* 1) Department of Surgery; 2) Obstetrics & Gynecology; 3) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO 63110.

Loss of DNA mismatch repair leading to microsatellite instability (MSI) is seen in ~28% of endometrial cancers. Epigenetic silencing of MLH1 is the most common cause of defective DNA mismatch repair. In our large series of endometrial carcinomas, >20% have MLH1 promoter methylation. We previously showed MLH1 methylation is associated with increased age at diagnosis. To date, no genetic factors associated with MLH1 methylation have been identified. We hypothesized that variation in DNMT genes might contribute to risk for MLH1 methylation. We undertook a case-control study to test for association between DNMT variants and abnormal MLH1 methylation. Five SNPs in each of DNMT1, -3A, -3B and -3L were typed in the normal DNA from women with endometrial carcinoma. Tumors were assessed for MSI and MLH1 methylation and clinical data were extracted from patient charts. For these studies, cases are women with cancers with MLH1 methylation (N=97) and controls are women with cancers without MLH1 methylation (N=220). Cases and controls were matched for race and tumor histology. Patient age and body mass index (BMI) were significantly associated with MLH1 methylation. Two DNMT3L SNPs, rs2070565 and rs968447, were also associated with the methylation phenotype (P=0.019 and P=0.003, respectively, logistic regression analysis including patient age and BMI). A risk haplotype for the rs968447 and rs2070562 DNMT3L SNPs was over-represented in cases (P=0.003). Analysis of 335 cancer-free controls from the St. Louis region (women >65 years of age with no history of cancer) confirmed DNMT3L SNP frequencies were different in women with endometrial cancers with MLH1 promoter methylation. This is the first report of association between variants in a DNMT gene and somatic epigenetic inactivation of MLH1. Identification of risk alleles for MLH1 methylation could shed light on mechanisms of epigenetic silencing and may lead to new approaches to the prevention of malignancies associated with MLH1 inactivation.

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Identification of the dyslexia susceptibility gene for DYX5 on chromosome 3. *J. Kere[1], K. Hannula-Jouppi[2], N. Kaminen-Ahola[2], M. Taipale[3], R. Eklund[2], J. Nopola-Hemmi[4], H. Kääriäinen[5].* 1) Department of Biosciences at Novum, Karolinska Institutet, Huddinge, Sweden; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) EMBL, Gene Expression Programme, Heidelberg, Germany; 4) Department of Pediatrics, Jorvi Hospital, Espoo, Finland; 5) Department of Medical Genetics, University of Turku, Turku, Finland.

Dyslexia, or specific reading disability, is the most common learning disorder with a complex, partially genetic basis, but its biochemical mechanisms remain poorly understood. A locus on chromosome 3, DYX5, has been linked to dyslexia in one large family and speech-sound disorder in a subset of small families. We found that the axon guidance receptor gene ROBO1, orthologous to the Drosophila roundabout gene, is disrupted by a chromosome translocation in a dyslexic individual. In a large pedigree with 21 dyslexic individuals genetically linked to a specific haplotype of ROBO1 (not found in any other chromosomes in our samples), the expression of ROBO1 from this haplotype was attenuated in affected subjects. Sequencing of ROBO1 in apes revealed multiple coding differences, and we observed significantly different evolutionary rates between orangutan on one hand and human, chimpanzee and gorilla on the other hand. We also identified novel exons and splice variants of ROBO1. We conclude that dyslexia may be caused by haploinsufficiency for ROBO1 in rare families. Thus, our data show that neuronal axon crossing across the midline between brain hemispheres, dendritic guidance, or another function of ROBO1 is a developmental process contributing to reading ability.

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Evolution and distribution of haplotypes in the complement factor H (HF1/CFH) gene that predispose to age-related macular degeneration. *M. Dean[1], B. Gold[1], J. Bergeron[2], D.H. Anderson[3], L.V Johnson[3], R. Allikmets[4], G. Hageman[5].* 1) Lab Genomic Diversity, NCI-FCRDC, Frederick, MD; 2) SAIC-Frederick, Frederick, MD; 3) University of California, Santa Barbara, CA; 4) Columbia University, New York, NY; 5) University of Iowa, Iowa City, IA.

Age-related macular degeneration (AMD) is the most frequent cause of irreversible blindness in the elderly in developed countries, affecting more than 50 million individuals worldwide. Our previous studies implicated activation of the alternative complement pathway in the formation of ocular drusen, the hallmark lesion of AMD, and demonstrated that the Factor H protein, the major inhibitor of the alternative complement pathway, accumulates within drusen. We also demonstrated that specific haplotypes in the Factor H gene (HF1/CFH) are highly associated with AMD, and may contribute as much as 50%; to the risk of this disease. Haplotype analysis revealed that multiple CFH variants confer either an elevated or a reduced risk of AMD.

Here we show using haplotype network analysis that the protective haplotypes are closely related to the ancestral haplotype and that the major risk haplotype is the most distantly related. The major haplotype determining variant in this gene (Y402H) displays extensive variation across samples from the Human Genome Diversity Panel, with high frequency in African, European, and Middle Eastern population, low frequency in most Eastern Asian groups and very low to absent in Native North and South American populations. The risk haplotype is also of low frequency (20%) in the Rapa Nui population of Easter Island, a group with an extremely low frequency of AMD. Several populations including those of Orkney Island (Scotland) Biaka Pygmy (Central African Republic) and Burusho (Pakistan) have very high frequencies of the Y402H allele (42-69%). These data indicate that variation in the distribution of CFH haplotypes are an important factor determining the incidence of AMD in world populations.

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The effect of common variants of the glucokinase gene on fasting blood glucose differs in pregnant and non-pregnant subjects. *M.N. Weedon[1], A.T. Hattersley[1], B. Shields[1], B. Knight[1], T. Wilkin[1], L. Voss[1], B. Metcalf[1], G. Davey-Smith[2], Y. Ben-Shlomo[2], T.M. Frayling[1].* 1) Peninsula Medical School, Exeter, UK; 2) Department of Social Medicine, Bristol, UK.

Glucokinase is a key regulator of fasting glucose. Monogenic mutations in *GCK* result in raised fasting blood glucose (FBG) and increased offspring birth weight (BW). It is not known how common *GCK* variants affect FBG and BW.

We determined the LD pattern across *GCK* and flanking regions (116kb) by re-sequencing 84 dbSNPs in 43 unrelated individuals. 23 tagging SNPs (tSNPs) captured 71% of SNPs at r^2 0.8 and 92% of SNPs at r^2 0.5. We genotyped these SNPs in 781 normal glucose tolerant fathers from the Exeter Family Study (EFS). SNPs with a P 0.10 for FBG association were genotyped in two further NGT cohorts (Plymouth N=301; Barry-Caerphilly Growth N=606).

Two tSNPs in the 5 *GCK* region were reproducibly associated with FBG: GCK(-30), MAF 18%, FBG increase of 0.06 mmol/l for GA/AA versus GG subjects (combined P=0.0001); and rs3757840, MAF 47%, FBG increase of 0.09 mmol/l (combined P=0.00002). GCK(-30) and rs3757840 are in LD (r^2=0.2,D=1). Regression analysis demonstrates that the GCK(-30) effect is explained by rs3757840 in NGT subjects.

We then went on too determine the impact of these 2 SNPs in pregnancy. GCK(-30) was strongly associated with FBG in 755 28-week pregnant females from the EFS (0.075mM, P=0.003), but, in contrast, rs3757840 was not (0.025mM, P=0.44). In pregnant females, therefore, GCK(-30) is associated with FBG independently of rs3757840. Birth weight data support this finding: using 2689 mother/child pairs we have shown that maternal, but not fetal, GCK(-30) genotype is associated with offspring BW (64g, P=0.001); conversely, maternal rs3757840 genotype is not associated with offspring BW.

In conclusion, we have demonstrated that variants upstream of the glucokinase gene are associated with fasting blood glucose and birth weight. The effect of these SNPs is dependent on pregnancy status, and is an example of gene-environment interaction.

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Variant of a gene located on chromosome 10q confers risk of type 2 diabetes mellitus. *S.F.A. Grant[1], G. Thorleifsson[1], I. Reynisdottir[1], R. Benediktsson[2,3], A. Manolescu[1], J. Sainz[1], H. Stefansson[1], V. Emilsson[1], A. Helgadottir[1], U. Styrkarsdottir[1], M.P. Reilly[4], D.J. Rader[4], Y. Bagger[5], C. Christiansen[5], V. Gudnason[2], G. Sigurdsson[2,3], U. Thorsteinsdottir[1], J.R. Gulcher[1], A. Kong[1], K. Stefansson[1].* 1) deCODE Genetics, Reykjavik, Iceland; 2) Icelandic Heart Association, Reykjavik, Iceland; 3) Landspitali-University Hospital, Reykjavik, Iceland; 4) University of Pennsylvania Health System, Philadelphia, USA; 5) Center for Clinical and Basic Research A/S, Ballerup, Denmark.

We previously reported suggestive linkage of type 2 diabetes mellitus (T2D) to chromosome 10q. The 10q linkage region has also been observed in Mexican Americans. Two hundred and twenty eight microsatellite markers were genotyped in Icelandic T2D patients and controls throughout a 10.5Mb interval. One of these microsatellites was associated with T2D ($P=2.1\times10^{-9}$). This was replicated in a Danish cohort ($P=2.4\times10^{-3}$) and a US cohort ($P=1.7\times10^{-9}$). Heterozygous and homozygous carriers of the at-risk alleles, 38% and 7% of the population respectively, have relative risks of 1.45 and 2.39 compared to non-carriers. This corresponds to a population attributable risk of 21%. The marker is located in the intron of a gene and is within a well defined LD block (based on CEPH Caucasian HapMap v16) that contains no additional genes. The gene encodes for a protein that resides within a pathway that has a potential role in T2D. In conclusion, these data in three populations constitute strong evidence and support of the notion that variants of this gene contribute to the risk of T2D. The identity of the gene and the corresponding data will be presented.

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Polymorphisms at the Chromogranin A and B Loci are Risk Factors for Hypertensive End-stage Renal Disease in African Americans. *R.M. Salem, P.E. Cadman, G. Wen, B.K. Rana, D.W. Smith, E. Eskin, M. Stridsberg, H.J. Ward, L. Taupenot, N.J. Schork, D.T. O'Connor.* Dept of Medicine, UCSD, La Jolla, CA.

Background: Chomogranins A (*CHGA*) and B (*CHGB*), stored with catecholamines, are differentially expressed in genetic hypertension. Since hypertensive end-stage renal disease (HT-ESRD) is highly prevalent in African Americans, we tested whether common polymorphisms at *CHGA/B* predict HT-ESRD. Methods: We genotyped 24 *CHGA/B* SNPs in n=58 blacks with HT-ESRD, and n=150 controls. Genotype/haplotype frequencies were compared between HT-ESRD and controls. Plasma levels of product peptides were measured. To probe the role of CHGA in catecholamine storage vesicles, expression was silenced in chromaffin cells by siRNA targeting of *Chga*. Results: Three genomic regions were associated with HT-ESRD: 2 at *CHGA* and 1 at *CHGB*. In *CHGA* promoter region G-462AT-415CC-89A, ATC was more frequent in the HT-ESRD group (OR=2.42; 95% CI: 1.04-5.67). *CHGA* haplotype spanning a portion of the coding region: G8540C[Glu246Asp]C9610T[Arg381Trp]G12602C, contained 2 haplotypes acting in opposing fashion: CCG conferred risk of HT-ESRD (OR=3.65; 95% CI: 1. 49-8.92), while GCC was protective (OR=0.22; 95% CI: 0.07-0.72). In *CHGB* region C10501TA11727G[Glu348Glu], the CA was protective (OR= 0.49; 95% CI: 0.24-0.98). Coding haplotypes of *CHGA* associated with a proximate biochemical phenotype (p=0.024): a progressive rise in $CHGA_{116\text{-}439}$ from the protection haplotype (GCC) to the risk haplotype (CCG) group. Thus, risk haplotype CCG activated gene expression *in vivo*. A similar trend was seen with *CHGB*. Application of *Chga* siRNA caused disappearance of chromaffin granules; dense-core granules per cell XY cell plane declined by ~2/3 (p0.0001). Conclusion: Common variants at both *CHGA/B* associate with risk of HT-ESRD. These haplotypes also associated with altered plasma levels of *CHGA/B*, consistent with augmented chromaffin granule biogenesis and sympathetic hyperactivity by risk variants. The results indicate that common genetic variants in the catecholamine storage and release pathway confer risk of HT-ESRD, and suggest novel approaches for prediction, diagnosis, and treatment of this disorder.

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A whole-genome admixture scan finds a candidate locus for multiple sclerosis susceptibility (LOD=5.2). *D. Reich[1], N. Patterson[2], P.L. De Jager[2,3], G.J. McDonald[1,2], A. Waliszewska[1,2,3], A. Tandon[1,2,3], R.R. Lincoln[4], C. DeLoa[4], S.A. Fruhan[1,2,3], P. Cabre[5], O. Bera[5], G. Semana[6], M.A. Kelley[7], D.A. Francis[7], K. Ardlie[8], O. Khan[9], B.A.C. Cree[4], S.L. Hauser[4], J.R. Oksenberg[4], D.A. Hafler[2,3].* 1) Dept Genetics, Harvard Univ, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 3) Laboratory of Molecular Immunology, Center for Neurologic Disease, Brigham and Womens Hospital, Boston, MA; 4) Department of Neurology, University of California, San Francisco, CA; 5) Clinique Neurologique, LHôpital Quitman, Fort-de-France, France; 6) Laboratoire Universitaire dImmunologie, University de Rennes 1 and Etablissement Français du Sang Bretagne, Rennes, France; 7) Department of Medicine, Division of Medical Sciences, University of Birmingham, UK; 8) Genomics Collaborative, Division of SeraCare Life Sciences Inc, Cambridge, MA; 9) Multiple Sclerosis Center, Depart.

Multiple sclerosis (MS) is a common disease with proven heritability in families that has resisted large-scale attempts to identify risk genes. Traditional linkage scans have so far identified only one risk haplotype for MS, but this explains only a fraction of the increased risk to siblings. Association scans such as admixture mapping have much more power in principle to find the weak factors that must explain most of the risk. We describe the first high-powered admixture scan, focusing on 605 African Americans with MS and 1,043 African American controls, and find a locus that is significantly associated to MS on chromosome 1 (LOD = 5.2). The signal is present in cases, but not controls, and grows stronger after follow-up genotyping. The apparent success with MS suggests that admixture mapping may be a practical way of finding disease genes more generally, and lends momentum to its application, in high-powered studies with more than two thousand cases each, to other diseases where it has the best prospects of working, to assess how useful it will be as a disease-gene finding method.

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Clustering of autoimmune diseases in multiple sclerosis (MS) families: evidence for genetic influences. *L.F. Barcellos[1,2], B. Kamdar[2], P.P. Ramsay[1], R.R. Lincoln[2], S. Schmidt[3], G. Artim[1], S. Caillier[2], C. DeLoa[2], M.A. Pericak-Vance[3], J.L. Haines[4], J.R. Oksenberg[2], S.L. Hauser[2].* 1) Univ California, Berkeley; 2) Univ California, San Francisco; 3) Duke Univ Medical Center; 4) Vanderbilt Univ Medical Center.

A large body of research supports the hypothesis that autoimmune mechanisms play a major role in MS pathogenesis. Familial clustering of multiple autoimmune diseases has been reported, and a careful examination of associations between diseases may provide important clues to defining common etiologic pathways. We determined the prevalence of co-existing autoimmune phenotypes in MS cases and first degree relatives. Comprehensive data was collected from 176 MS families (1317 first degree relatives, 386 MS cases). A total of 26% of MS index cases (1 per family) reported at least 1 coexisting autoimmune disorder. The most common conditions present were Hashimoto thyroiditis (HT) (9.7%) and psoriasis (5.6%). A total of 105 (60%) MS families reported at least 1 first degree relative with an autoimmune condition, whereas 71 families (40%) had no history of autoimmunity other than MS. As observed in cases, HT and psoriasis were the most prevalent conditions in family members. In fact, index cases diagnosed with HT and/or psoriasis were more likely to have first degree relatives with these same conditions (OR=3.4, p=0.003). Genome screening (390 markers) and nonparametric mpt analyses was performed in families with these 2 additional diseases (n=64). Chr 16 had the highest score (D16S764; MLOD=2.80); MS families in which no HT or psoriasis was present (n=112)showed no evidence for linkage to this region (MLOD=0.05).In addition, a common variant within CTLA4, a proposed autoimmunity gene, was strongly associated in MS families with other autoimmune diseases (p=0.008).Our results confirm that other autoimmune conditions are present in MS cases and family members, and suggest that certain diseases are associated with MS. In addition, we propose that different MS phenotypes may exist which are defined by the presence/absence of other autoimmune diseases, and for which susceptibility may be conferred by different genetic factors.

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Polymorphisms in genes from the interferon signaling pathway are associated with systemic lupus erythematosus. *S. Sigurdsson[1], G. Nordmark[1], H. Göring[2], M-L. Eloranta[3], G. Alm[3], L. Rönnblom[1], A-C. Syvänen[1].* 1) Department of medical sciences, Molecular Medicine, Uppsala, Uppsala, Sweden; 2) Dept of Genetics, Southwest Foundation for Biomedicial Research, San Antonio, Texas, USA; 3) Dept of Molecular Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease caused by both genetic and environmental factors. We have previously, using SNP genotyping and joint linkage and association analysis dissected the function of the type I IFN system in SLE. In two genes, the tyrosine kinase 2 (TYK2) and interferon regulatory factor 5 (IRF5) genes, we identified SNPs that displayed strong signals of joint linkage and association ($p < 10E-7$) with SLE in the Swedish, Finnish and Icelandic populations. (Sigurdsson S. et al. 2005, Am J Hum Genet 76:528). Haplotype association analyses of multiple SNPs in both genes revealed no association with SLE with higher statistical significance than analysis of the individual SNPs. We are now performing functional studies on the consequences of the identified SNPs. The TYK2 SNP alters an amino acid in a domain of TYK2 that interacts with the type I IFN receptor, and is required for maintaining expression of the receptor on cell membranes. Transfection analyses in TYK2 deficient cells indicate that the TYK2 SNP alleles play a functional role in the type I IFN signaling system. The IRF5 SNP is the first nucleotide of intron 1 of the gene, and could be functional by causing alternative splicing. Accordingly, we observe complex splicing patterns in RNA samples from 44 SLE patients collected so far. Both our genetic and functional data support a disease mechanism in SLE that involves key components of the type I IFN system. Consequently, we are also performing a large scale genetic linkage and association study of 1500 SNPs in over 100 genes from the type I IFN system using the Illumina genotyping platform available in our laboratory.

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Association of the Regulatory Regions of *OX40L* with Systemic Lupus Erythematosus (SLE). *D.S. Cunninghame Graham[1], A.M. Richardson[2], T.W. Behrens[3], J.D. Rioux[2], T.J. Vyse[1].* 1) Imperial College, LONDON, UK; 2) Broad Institute, MIT, Boston, USA; 3) University of Minnesota, Minneapolis, USA.

Purpose: SLE is a complex autoimmune disease trait of uncertain aetiology. Evidence supports a genetic contribution to disease susceptibility. *OX40L (TNFSF4)* was chosen as candidate susceptibility gene for SLE in this family-based association study, because of its important role in T-B cell interaction. *OX40L* (1q25), is in a region identified in two genome-wide linkage scans. Additionally, 1q24 is homologous to a region on mouse distal chromosome 1 important in murine models of SLE. *OX40L* provides a co-stimulatory signal to T cells and may play a role in the migration of lymphocytes to sites of inflammation through its expression on the surface of endothelial cells. **Methods:** The study cohort consists of 474 SLE European Caucasian families, from a total collection of 630 SLE families. 33 variants in *OX40L* were identified from SNP databases or by re-sequencing typed by MALDI-TOF mass spectrometry or PCR-RFLP. Initial haplotype maps in SLE trios were constructed in Haploview. TDT analysis, on both single SNPs and on haplotypes, was performed using GENEHUNTER on trios and TRANSMIT to include single parent families. **Summary:** There are two clear haplotype blocks across *OX40L*. TDT analysis for SNPs in the 5 block, showed a significant over-transmission for the rare G allele of SNP6 in the promoter (p=2.7E-05). This was corroborated in an independent collection of ~ 450 US SLE families (p=0.02). The 3 haplotype block shows an independent genetic effect, but is more heterogenous. There were different associated alleles in the two populations, but the UK over-transmitted haplotype carried the over-transmitted alleles in the US population. **Conclusion:** In the 5 haplotype block for *OX40L* we have corroborated an association with SNP6, a variant predicted to disrupt the binding site for Upstream Stimulatory Factor. In the 3UTR, the associated SNP may play a role in mRNA stability. We are currently investigating the functional significance of both of these variants. Increased transcription and/or mRNA stability may increase the co-stimulatory signal and hence immune response.

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Genetic association of celiac disease to myosin IXb points to an intestinal barrier defect as an early pathogenic event. *C. Wijmenga[1], P.I. de Bakker[2], B.Z. Alizadeh[1], M. Daly[2], M.R. Bevova[1], M.C. Wapenaar[1], B.P. Koeleman[1], A.J. Monsuur[1].* 1) Complex Genetics Section, Univ Medical Center, Utrecht, the Netherlands; 2) Broad Institute of M.I.T. and Harvard, Cambridge, MA 02142, USA.

Celiac disease is a chronic systemic autoimmune disease resulting from intolerance to gluten, a storage protein present in wheat, rye and barley. Ingestion of gluten leads to inflammation and damage of the small intestine, resulting in malnutrition and associated complications. With a prevalence of close to 1%, celiac disease is the most common food intolerance in general Western populations. It is strongly associated to HLA class II molecules that explain some 40% of the familial clustering. A previous study identified a novel susceptibility locus on 19p13.1 (CELIAC4 locus), and showed association to one of the microsatelite markers (D19S899) tested in the linkage screen. Recently, we tested 15 single nucleotide polymorphisms (SNPs) around D19S899 in two independent case-control cohorts and found significant association to variants in the myosin-IXb gene (MYO9B). The strongest signal was observed for an intronic SNP, (P = 2.1.10-6). The genotype frequency data for this SNP revealed an increased risk of celiac disease in both homozygote carriers (OR 2.27; CI 1.56 to 3.30) and heterozygote carriers (1.66; CI 1.23 to 2.13), suggesting a co-dominant effect of this MYO9B variant. In addition, a comprehensive screen with 269 SNPs covering the entire linkage region was performed to rule out that an association signal somewhere else in the CELIAC4 region had been overlooked. This comprehensive screen revealed a single peak of association, in the MYO9B gene with one SNP showing strong significance (P = 7.8.10-5) after stringent Bonferroni correction. This SNP is in strong LD with the SNPs identified in the previous analysis. With regard to its function, MYO9B may point to an underlying intestinal barrier abnormality as a primary etiological event. Hence, this gene may be one of the long-sought factors involved in the early mucosal events preceding the well-understood inflammatory response. Importantly, this gene may be relevant for other autoimmune disorders with enhanced intestinal permeability.

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Loss of imprinting of IGF2, H19, PEG1/MEST and PEG3 in Head and Neck Cancers. *H. Kataoka[1], S. Nakano[2], M. Oshimura[2], H. Kitano[1].* 1) Otolaryngology, Head and Neck Surgery, Tottori University, Tottori, Japan; 2) Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, Tottori University, Tottori, Japan.

INTRODUCTION: Genomic imprinting is an epigenetic feature that may play a significant role of tumorigenesis. Previous studies showed that abnormal imprinting was observed in the human chromosomes 11p15.5 region containing H19, IGF2 and p57KIP2 imprinted genes in head and neck cancer. However, few study regarding other imprinted genes in head and neck cancers was reported. Therefore, to further identify and to characterize genetic events associated with these carcinomas, we examined the imprinting status of several imprinted genes in 22 cases of head and neck carcinomas. MATERIALS AND METHODS: Head and Neck cancers, diagnosed by histopathologic examination, and matched normal tissues of 22 patients were analyzed. These consisted of 5 tongue carcinomas, 4 hypopharyngeal carcinomas, 2 oropharyngeal carcinomas, 9 laryngeal carcinomas, 2 thyroid carcinoma. Genomic DNA and RNA were isolated and allelic expression of the genes has been determined by PCR-RFLP utilizing restriction enzymes. Restriction enzymes were as follows: ApaI for IGF2, RsaI for H19, AflIII for MEST and MnlI for PEG3. RESULTS: We analyzed 22 primary head and neck carcinomas for the loss of imprinting in the IGF2, H19, PEG1/MEST and PEG3. After PCR amplification and endonuclease cleavage, informative case of H19, IGF2, PEG1/MEST and PEG3 showed 4, 2, 4 and 2 cases, respectively. LOI of H19, IGF2, PEG1/MEST and PEG3 were observed in 2, 1, 4 and 1 cases, respectively. LOI of H19 and PEG3 were only observed in tumor specimens, while LOI of IGF2 and PEG1/MEST were observed in both tumor and proximal normal specimens. Conclusions: We detected the LOI of IGF2 and H19 as previously reported. In addition, we newly identified the epigenetic changes at PEG1/MEST and PEG3. These findings suggest that a number of imprinting genes play possible roles of oncogenesis of head and neck cancer. Further studies are necessary for their biological significance and prognostic parameter and cancer predisposition of LOI in normal tissues.

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Refinement of critical regions of 7q deletions in uterine leiomyomas using high-resolution microarray CGH. *N.C. Wortham[1], S. Vanharanta[2], C. Langford[3], W.F. Bodmer[4], L.A. Aaltonen[2], I.P.M Tomlinson[1].* 1) Molecular & Population Genetics Laboratory, Cancer Research UK, London, United Kingdom; 2) Dept of Medical Genetics, Biomedicum Helsinki, PO Box 63 (Haartmaninkatu 8), FIN-00014 University of Helsinki, Finland; 3) Microarray Facility, Wellcome Trust Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; 4) Cancer and Immunogenetics Laboratory, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.

Deletions involving 7q are common cytogenetic events in uterine leiomyomas, occurring in approximately 25% of cases. Current techniques involving micorsatellite-based loss-of-heterozygosity analysis have demonstrated several different minimal regions in 7q22.2-7q22.3. We have constructed a high-resolution microarray of 1083 overlapping BAC clones from 7q to further refine this region, and to define deletion breakpoints. We have refined the region to approximately 2.5Mb in 7q22.3-q31.1. Furthermore, we have identified a novel region of deletion in 7q34, and have refined this to approximately 2.5Mb.

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Differential Expression of Adhesion Molecules in H460 Human Non-small Cell Lung Cancer Cells and the Paclitaxel Resistant Mutant H460/TAX. *L. Tan Chen[1], Y.C. Lin[2], J.J. Chu[3].* 1) Department of Medical Technology, Chung Tai Institute of Department of Medical Technology, Chung Tai Institute of Health Sciences and Technology ,Taiwain; 2) College of Veterinary Medicine, Nation Chung Hsing University,Taiwain; 3) Division of Chest Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, ROC.

The purpose of this study was to fine the integrins in differential _expression of adhesion molecules in H460Human Non-small Cell Lung Cancer Cells (NSCLC) and the Paclitaxel Resistant Mutant H460/TAX. Paclitaxel is among the most promising new agents in the treatment of advanced NSCLC. Most patients with advanced NSCLC initially respond to paclitaxel (30% response rate); however, overall survival has not improved in part because of the development of acquired paclitaxl resistance. The best understood mechanism paclitaxel resistance is the expression of the P-glycoprotein (Pgp), encoded by mdr1 gene, which is responsible for the multidrug resistance (MDR phenotype). Pgp-mediated resistance to paclitaxel is characterized by cross-resistance to many structurally and functionally distinct anticancer agents, including anthracycline (doxorubicin and epirubicin), Vinca alkaloids (vincristine and vinblastine), epipodophyllotoxins, and camptothecins. CD44, CD29,CD49b,CD49d,CD4949f,CD54,CD56,CD106.

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How to Use the Online NCI/NCBI Entrez Cancer Chromosomes Search Database. *T. Knutsen[1], V. Gobu[2], T. Ried[1], K. Sirotkin[2].* 1) Genetics Branch, NCI/NIH, Bethesda, MD; 2) National Center for Biotechnology Information, NIH, Bethesda, MD.

The NCI/NCBI Entrez Cancer Chromosomes database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=cancerchromosomes) was developed as a part of the NCI Cancer Chromosome Aberration Project (CCAP) initiative to integrate the cytogenetic and physical maps of the human genome and to identify new cancer-associated genes. It integrates the SKY/M-FISH & CGH Database, the Mitelman Database of Chromosome Aberrations in Cancer, and the Recurrent Chromosome Aberrations in Cancer databases. All three databases are searched simultaneously, and seamlessly, and a variety of queries can be performed to track specific abnormalities and relate them directly to map and sequence data through the Map Viewer database. CGH abnormalities can be directly compared to karyotypic aberrations. All cases are directly linked to their PubMed abstracts. Searching can be done a variety of ways, including the Entrez standard free text search, and customized fielded searches. Search results can be viewed as: (1) a "summary" report that shows the number of cases in each database matching the query, with an abstract for each case giving a partial karyotype, diagnosis, and links to the full karyotype, CGH profile, clinical information, and PubMed abstract; (2) a "detailed" report that lists all the cytogenetic aspects of each case and reference information; and (3) a "similarity" report that lists all affected diseases, sites, chromosomal breakpoints, junctions, numerical abnormalities, and bands gained/lost. The similarity report presents both the textual and cytogenetic elements with a count (number) that links to those cases containing that element. For example, if a similarity report is performed on a set of cases where 59 of 80 have a breakpoint at 11p12, then "59" will be displayed by that breakpoint, and by clicking on "59," the user will go to a page displaying summary reports for those 59 cases. Cases related textually or genetically are linked from all the input databases. This presentation will demonstrate how to make use of the advanced and innovative features of the database and its interface, including the ability to discover similar genetic features from a set of cases.

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Computational aspects of large cytogenetic data collections. *M. Baudis*[1,2]. 1) Dept. of Pediatrics, U of Florida, Gainesville, FL; 2) UF Shands Cancer Center, Gainesville, FL.

Over the last decades, thousands of (molecular-) cytogenetic studies have led to the description of karyotype abnormalities in tumor cells. In agreement with the multistep model of oncogenesis, repetitive chromosomal aberration patterns may reflect the cooperation of different genes in most malignant diseases. A systematic analysis of these patterns for oncogenomic pathway description requires the large scale compilation of (molecular-) cyogenetic tumor data, and the development of specific tools for cytogenetic data mining purposes. Major questions addressed by computational analysis of large scale cytogenetic data collections are:

Do complex cytogenetic aberration patterns reflect disease specific mechanisms, or are they signs for general co-operative mechanisms during tumor development?

Do non-random genomic aberration patterns relate to specific genes relevant for the development of the corresponding tumor type?

As part of the Progenetix project, a collection of cytogenetic data from human neoplasias has been made publicly available. Currently, results from more than 12,000 predominantly CGH experiments are accessible in a format suitable for standard data mining techniques. In limited datasets, cytogenetic aberrations have been successfully utilized by us and others for deliniating clinically relevant disease subentities. Also, the construction of sequential and forked models for the accumulation of specific cytogenetic changes has been attempted. However, the global view on tens of thousands of tumor cases available through the Mitelman database and Progenetix requires new models which take into account the high diversity of possible genomic hot spot regions. In our current view, emphasis is being put on the correct identification of relevant genomic intervals with a high probability of non-random change. Here, an overview of the data sources as well as a presentation of our current large scale data mining attempts will be presented.

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The Expression of CD56 is Highly Increased in a Paclitaxel-resistant Human Non-small Cell Lung Cancer Cell Line. *T.C. Lai[1], J.J. Chu[2], J.Y Hsu[2].* 1) Department of Medical Technolo, Chung Tai Institute of Health Sciences and Technol, Taichung,, Taiwan; 2) Division of Chest Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, ROC2.

Drug resistance to paclitaxel is a major problem during cancer chemotherapy. For study the cellular and molecular mechanism, we have established a paclitaxel resistant mutant cell line called H460/TAX derived from human non-small cell lung cancer (NSCLC) H460 cells (Chu et al., Anticancer Res. 20: 2449-56., 2000). Recently, we found the cell adhesion activity of H460/TAX is different from that of the parental H460. We were thus interested in identifying any alterations in the expression of adhesion molecules. Using immunofluorescence staining and flow cytometer, a variety of adhesion molecules were monitored, such as integrins, ICAMs, VCAMs, NCAMs. Among them, we found that the expression of CD56 was dramatically elevated in H460/TAX as compared with that of H460. CD56 is a member of the family of neural cell adhesion molecules expressed on the surface of tumor cells of neuroendocrine origin, including small cell lung cancer (SCLC), carcinoid tumors, neuroblastomas, and neuroectodermal tumors such as astrocytomas. It is also cross-reactive with cardiac, neurologic, and neuroectodermal tissue as well as natural killer T cells. To our information, there are no previous studies reported the relation between CD56 and drug resistance on NSCLC. The study of genetic regulation of CD56 in both H460 and H460/TAX is also carry out in our laboratory.

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Complex translocation (8;21;2): a new variant of translocation (8;21) in acute myeloid leukemia. *J. Xu[1], S. Nan[1], D. Munavish[1], I. Chin-Yee[2], R.M. Barr[2], L. Minuk[2], A. Xenocostas[2].* 1) Cytogenetics; 2) Hematology, London Health Sciences Centre, University of Western Ontario, Canada.

In acute myeloid leukemia (AML), the standard t(8;21)(q22;q22) which generates the ETO/AML1 fusion protein is generally associated with favorable outcomes. Variants of t(8;21) involving other chromosomes account for approximately 3% of t(8;21) in AML cases. Because of rare occurrences, the clinical significance of these variants is yet to be established. We report the first case of t(8;21;2) associated with AML. The patient is a 31-year-old male who presented with one month history of progressive fatigue and dyspnea. His past medical history includes obstructive sleep apnea and a seizure disorder. His physical examination was normal with no evidence of lymphadenopathy or organomegaly. The blood count showed a WBC of 2.3X109/L with 31% blasts, neutropenia, anemia, and thrombocytopenia. The bone marrow was hypercellular with a marked increase in poorly differentiated blast cells (80%), histologically consistent with the AML-M1 subtype. Flow cytometry demonstrated that blasts expressed CD34, CD33, CD13, CD117, HLA-DR, myeloperoxidase, and dim cytoplasmic CD79a, confirming the myeloid origin. Of 25 G-banded metaphase cells examined, 24 cells had 45,X,-Y,t(8;21;2)(q22;q22;p21) and one cell had 46,XY. FISH showed 89% (177/200) of the cells with ETO/AML1 fusion signals on derivative chromosome 8, der(8)t(8;21). The patient is currently undergoing induction chemotherapy. More case reports are needed for understanding of diagnostic and prognostic implications of such variants.

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A Rarity of del(16)(q22) in an Acute Promyelocytic Leukemia (FAB AML M3v) with Classic Cytogenetic Alteration (15;17)(q22;q21). *J.H. Lin[1,2], E. Suguerra, Jr.[1], M. Zhuang[1], A. Karnik[1], H.O. Shah[1,2].* 1) Pathology & Labs, Nassau Univ Medical Ctr, East Meadow, NY; 2) State Univ. New York at Stony Brook, Stony Brook, NY.

A 39 year-old woman underwent a maxillary sinus antrostomy for sinusitis, epistaxis and nasal polyps. Blood tests revealed WBC of 0.9x103/mm3 with 74% immature cells, 11% segmented neutrophils, 12% monocytes and 4% lymphocytes. The RBC was 2.19x106/mm3, Hb 6.6 gm/dL, Hct 20%, MCV 91.5 fl and platelets of 36x103/mm3. The marrow specimens showed rather similar findings with cellularity approaching 100%. The immature cells display reniform nuclei with occasional nucleoli and fine granules and rare delicate Auer rods in cytoplasm. The acute granulocytic leukemia FAB AML M3v (APL) was confirmed and the chromosome studies showed 46,XX,t(15;17)(q22;q21)[7]/46,XX.t(15;17)(q22;q21) del(16)(q22)[13]. The patient was treated with trans retinoic acid (ATRA) and corticosteroids followed by scheduled chemotherapy and is doing fine one year later. The conventional APL are known to exhibit PML and RARA genes fusion giving rise to the concept of treating APL with retinoic acid compounds with good initial response. Chromosome 16 alterations i.e. inv16, 16q- and del(16)(q22) and del(16)(q11) etc. have frequently been reported in FAB AML M4e, M5a and M1. A combined (15;17)(q22;q11) and del(16)(q22) alteration is extremely rare. Two cases have been reported and one of which has been reported as M6 with a complex karyotype involving chromosomes 1, 2, 11 and 17, which on a subsequent relapse, transformed to M3. Fusion of the gene CBFB in 16q22 with MYHII in 16p13.1 can apparently render a favorable prognosis. Clonal evolution or disease modification may be associated with del(16)(q22), yet the exact mechanism by which these chromosomal changes exert in the pathogenesis related to M3 deserves further studies. Furthermore, if there is any residual del(16)(q22) re-exerting its influence on the clonal transformation to induce secondary leukemia other than M3 remains speculative.

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A CASE REPORT: PATIENT WITH IMMUNOSUPRESION PATTERNS, CLINICAL CARDIOPULMONARY MALFORMATION AND CELL PROLIFERATION, IN RELATION TO 11;14 TRANSLOCATION (47,XY, t(11;14) (q25;q24.1), +der(14) t(11;14) (q25;q24.1). *J.M. Aparicio[1,8], N.C. Gil-Orduña[2,8], M. Barrientos-Pérez[3], L. Pérez-Ricardez[4], R. Maldonado-Alonso[5], W.B. San Martín[2], F. Mezquita-Lopez[2], T.K. Carral-Valdez[2], M.L. Hurtado Hernandez[6], M. Gil-Barbosa[7], E. Huitzil[8], C.F. Salinas[9].* 1) Medical Genetics; 2) Estomatology; 3) Endocrinology; 4) Infectology; 5) Cardiology; 6) Citogenetics; 7) Direction, Hospital para el Nino Poblano, Puebla, Mexico; 8) Estomatology, Benemerita Universidad de Puebla, Mexico; 9) Craneofacial Genetics, University of South Carolina, U.S.A.

INTRODUCTION. Translocation t(11;14) has been observed in cell proliferation as mantle cell lymphoma, plasma cell leukaemia, in chronic lymphocytic leukaemia, in multiple myeloma, immunosupresion, cardiovascular malformation and a caracteristic phenotipe. CLINICAL CASE. A Mexican newborn male with partial trisomy for the derivation of the distal part of the long arm of chromosome 14 (14q24 leads to qter) diagnosed at Pediatric Hospital in Puebla, Mexico. The anomaly was a balanced translocation t(11;14) (q25;q24.1). The clinical manfestations of the patient were, lobar neumony, hidrocele, septicemia, interauricular septal defect, cleft palate and a dismorfic phenotipe. CYTOGENETICS. Some chromosomal translocations has been reported as primary etiological factor for different kind of neoplasias. t(11;14) in relation to mantle cell lymphoma. And has reported the frequency of t(11;14): 50-70% in mantle cell lymphoma, 10-20% originating from pre-immune B-cells in the mantle zone of the primary follicles in secondary lymphoid organs, in plasma cell leukaemia, and in splenic lymphoma with villous lymphocytes, and 2-5% in chronic lymphocytic leukaemia, in multiple myeloma and also in immunosupresion. CONCLUSIONS. Two previous cases involving the same segment q25;q24 of both 11 and 14 translocated chromosome has been reported. The karyotype in this study was confirmed as 47,XY, t(11;14) (q25;q24.1), +der(14) t(11;14)(q25;q24.1). clinical signs and simptoms, cytogenetics aberration and the possible treatment for a better life quality were analized.

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A case of CD5+ diffuse large B-cell lymphoma in bone marrow with unusual chromosome abnormalities involving amplification of the BCL-2 gene. *S.R.S. Gottesman[1], M.J. Macera[2,3], J. Cheng[1], A. Falarieh[1], J. Breshin[2,3], R. Zeng[2,3], P. Chandra[3], A. Babu[2,3].* 1) Dept of Pathology, SUNY Downstate Medical Ctr, Brooklyn, NY; 2) Div of Molecular Medicine and Genetics; 3) Dept of Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY.

A 66 year old male presented with splenomegaly and fever of unknown origin. The laboratory findings were Hb 10.0 g/dl; Hct 29.5%; WBC 3.15 x109/L; Plts 89,000 x 109/L; LDH 2,090 U/L. A bone marrow biopsy showed a diffuse infiltrate ~ (20 %) of large regular cells that were CD5+, CD20+. Cytogenetic analysis of bone marrow metaphases, revealed two related abnormal clones, a stemline and a sideline with an additional abnormality, in addition to normal cells. The karyotypes were: 47,XY,del(6)(q15),+i(18)(q10).ish i(18)(wcp7-,wcp18+,BCL-2++)[11]/ 47,idem,der(14) t(14;18)(p11.2;q21).ish der(14)(wcp18+,BCL-2+)[3]/46,XY[6]. The IGH/BCL-2 gene rearrangement that is common in diffuse large B-cell lymphoma, was absent in this case. The dual fusion IGH/BCL-2 probe (Vysis) and wcp18 were applied to metaphase chromosomes. No IGH/BCL-2 rearrangement was found, however, the additional i(18)(q10) was shown to have two extra copies of BCL-2, resulting in 4 copies of BCL-2 in the stemline. The uncommon rearrangement, der(14)t(14;18)(p11.2;q21), also contained one more copy of the BCL-2 gene, contributing to a total of 5 copies of BCL-2 in the sideline. The argyrophilic nucleolar organizer regions (AgNORs) are located in the stalks of the five acrocentric chromosomes and are loops of ribosomal DNA associated with the proliferative potential of the cells. AgNOR staining of metaphases failed to show any silver located on the der(14) chromosome. Negative silver staining indicates lack of production of the argyrophilic NOR-related proteins; nucleolin, C23 protein, RNA polymerase I or B23 protein. The possibility remains that the regulatory sequences of the ribosomal cistrons are influencing BCL-2 production on the der(14). This is a case of BCL-2 gene amplification, rather than translocation, the classical cause of over expression in B-cell lymphoma.

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A new complex chromosomal translocation (12;21;2)(p13;q22;q33) in acute lymphoblastic leukemia. *E.A. Vásquez[1,2], J.P. Meza[4], M.L. Ayala[1], B. López[3], J.A. Morales[1,2], J.R. González[2].* 1) Doctorado en Genética Humana, Universidad de Guadalajara; 2) División de Genética, CIBO-IMSS; 3) Departamento de hematología, Hospital de Pediatría CMNO-IMSS; 4) fUnidad Academica de Ciencias de la Salud y Tecnologia. Facultad de Medicina de la Universidad Autonoma de Tamaulipas.

Introduction: Chromosomal translocation t(12;21)(p13;q22) fuses ETV6 (TEL) and AML1 (CBFA) genes located on 12p13 and 21q22, respectively. This translocation is present in about 25% of children with precursor-B acute lymphoblastic leukemia (ALL) and can not be detected by conventional cytogenetic methods. Objective: To describe a new complex chromosomal translocation in an ALL patient. Material and method: A 4-year-old girl was diagnosed with B-precursor ALL. Physical examination reveled bone pain, ganglions of big size, hepatomegaly and splenomegaly. Hematological data were as follows: hemoglobin 7.8 g/dL, leukocytes 4.1 X103 /mm3 with 82.4% lymphocytes and platelets 24,000/ mm3. Immunophenotype, karyotype and FISH were done in a bone marrow sample. Results: Immunophenotype of the blast cells was CD19+(all cells B and their precursors), CD20+(cells B but, not plasmatic cells) and CD22+(mature B cells but, not plasmatic cells). The karyotype was: 46,XX,t(2;12)(q33;p13)[2]/47,XX,t(2;12)(q33;p13),+21[2]/46,XX[12]. FISH detected the fusion of ETV6/AML1 genes. Sub-clones with +21 displayed the fusion ETV6/AML1 and two independent AML1 signals. Moreover, a small red signal corresponding to AML1 gene sequences was observed on the derivative 2. Discussion: Complex translocations associated with t(9;22) in chronic myelocytic leukemia are frequently observed. In contrast, they have rarely been observed linked to t(12;21). To our knowledge, there are only seven complex translocation related to the t(12;21) and ETV6/AML1 fusion. These complex rearrangements may result from two consecutive translocations. The prognosis for these variant translocations remains to be determined.

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A constitutional ins(6;7)(q23;q36q31)c in a patient with smoldering multiple myeloma. *R.A. Conte[1], L. Rajdev[2], D.T. Walsh[1], D. Wei[1], M. Zohouri[1], A.R. Deshikar[1], T. Zhou[1], C. Johnson[1], H. Ratech[1], V.R. Pulijaal[1], K.H. Ramesh[1], L.A. Cannizzaro[1].* 1) Dept. Pathology, Montefiore Medical Ctr/AECOM, Bronx, NY 10461; 2) Dept. Oncology, Montefiore Medical Ctr/AECOM Bronx, NY10461.

A 50 year-old male with a 4-year history of smoldering multiple myeloma (SMM) presented with fatigue, anemia and loss of appetite. SMM is a plasma cell dyscrasia where the disease is asymptomatic and is diagnosed by the finding of an elevated serum monoclonal-protein concentration (>3g/dL) during a screening examination, as was the case for this patient. The bone marrow was normocellular (50%) with a myeloid to erythroid ratio of 3:1. There were interstitial aggregates of plasma cells, some of them with Dutcher bodies, in the interstitium and constituted 50% of the overall cellularity. There were no granulomas. Stainable iron was reduced. The bony trabeculae were unremarkable. Immunohistochemical stain demonstrated that there were 50% CD138+ plasma cells, which were kappa-restricts, (plasma cells with Kappa light chain excess) consistent with plasma cell neoplasm. GTG banding and FISH analyses of unstimulated bone marrow followed by peripheral blood chromosome analysis revealed a: 46,XY,ins(6;7)(q23;q36q31)c.ish ins(6;7)(6ptel+,wcp6+,wcp7+,wcp6+,6qtel+; 7ptel+,wcp7+,D7S522+,wcp7+,7qtel+). FISH analysis using the following probes: 6ptel, 6 whole chromosome paint (WCP), 7 WCP, 6qtel, 7ptel and D7S522 confirmed the ins(6;7) and also that the 7q31 locus was still intact and present on the abnormal chromosome 7. This chromosomal rearrangement was determined to be constitutional in the patient when cytogenetic analysis of PHA stimulated peripheral blood was performed. It is interesting to note that the breakpoints involved in this genomic alteration are the putative locations of genes that are likely to play a role in leukemogenesis. The breakpoint on 6q and the proximal breakpoint on 7q apparently lie within the respective common regions of chromosome loss observed in hematological neoplasms, including multiple myeloma. Further molecular and clinical studies will help to shed light on a correlation between SMM and the genomic regions that play a predisposing developmental role.

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Comparison of interphase panel FISH and routine chromosome analysis results in patients with hematological malignancies. *M. Zohouri, D.T. Walsh, L.A. Cannizzaro, V.R. Pulijaal, R.A. Conte, D. Wei, A.R. Deshikar, T. Zhou, C. Johnson, K.H. Ramesh.* Dept Pathology, Montefiore Medical Ctr, Bronx, NY.

Conventional cytogenetic evaluation of G-banded chromosomes can be an important tool in establishing a diagnosis and prognosis in a number of hematological disorders. However, the utilization of an appropriate combination of fluorescence in situ hybridization (FISH) probes can give us valuable information when routine cytogenetic results are normal or when not possible. We employed 6 panels of FISH probes, based on clinical findings, to compare cytogenetic results of bone marrow (BM) or unstimulated peripheral blood (PBU) with interphase FISH results in 50 patients with suspected clinical diagnoses of myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM) or non-Hodgkins lymphoma (NHL) at Montefiore Medical Center, Bronx, NY. In 5 cases (10%), both routine chromosome analyses and FISH results were abnormal; in 19 cases (38%), both chromosome and FISH results were normal; 3 cases (6%) were abnormal by karyotype alone, and 10 cases (20%) were abnormal by FISH findings only. Additionally, of 13 cases where chromosome analyses had failed, FISH studies revealed normal results in 5 cases (10%) and abnormal results in 8 cases (16%). This study of 50 cases showed that in 36% of the cohort with normal cytogenetic results or where chromosome analyses were not possible, chromosome abnormalities were detected by panel FISH alone. These findings confirm that panel FISH analysis is a useful and powerful tool to detect disease specific chromosome abnormalities, and, when used in conjunction with conventional cytogenetic studies, can improve the detection rate of chromosome abnormalities in patients suspected of or having hematological malignancies. Performing panel FISH analysis not only provides a rapid diagnosis, but also has a significant impact on prognosis.

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Changing Signal Patterns - Fluorescent in- situ hybridization in monitoring CML. *Y. Ding, P. Koduru, S. Gupta.*
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New molecular cytogenetic techniques are being increasingly applied as routine investigative tool in hematological malignancies. The BCR-ABL gene rearrangement generated by Philadelphia translocation(Ph), t(9;22)(q34;q11.2), is the hallmark of chronic myeloid leukemia. The technique of fluorescent in -situ hybridization (FISH) is extensively applied at the initial diagnosis of CML,to investigate cases with failed cytogenetics,to detect cryptic BCR-ABL gene fusion and to monitor disease response. A deletion of prognostic significance encompassing ABL-BCR product from the derivative chromosome 9 occurs in 15% of CML cases, and is more common in patients with variant translocation. It may take place at the time of Ph chromosome formation, with progression of the disease or may appear at the time of relapse. Reports of physical separation of ABL-BCR in non-deleted patients as well as evolution from classical to variant translocation introduce further heterogeneity.The correct diagnosis using FISH technique in such setting is influenced by the experience and interpretational skills of the investigator and DNA probes used in the study. We present interpretation of change in FISH signal pattern observed using BCR and ABL dual color dual fusion translocation probe in a 45 years old patient with leukemia. Chromosome analysis of G-banded metaphase preparation showed classical Philadelphia translocation,t(9;22)(q34;q11.2), which was confirmed by FISH studies (I Green, 1 Red, 2 Fusion signals). Patient underwent chemotherapy for CML. During follow-up studies, chromosome preparations failed and FISH studies showed normal signal pattern (2R,2G) in 98-99% of interphase cells. Eight months after the diagnosis, an atypical signal pattern (1R,1G, 1F) was detected in 40% of interphase cells. Use of ASS gene probe confirmed deletion on derivative 9. RT-PCR was positive for BCR-ABL product. We suggest that atypical interphase FISH patterns should not be interpreted in isolation. Information gathered through more than one source, i.e. conventional cytogenetics, molecular cytogenetics and molecular genetics should be used collectively to interpret patient results.

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Sequential Genomic Changes in Chronic Lymphocytic Leukemia. *C. Wang, L. Zou, L. Li, J. Lian, R. O'Dell, M. Shaver, J. O'Malley, J. Defeo, N. Wang.* University of Rochester School of Medicine, Rochester, NY.

To define the sequential genomic aberrations associated with the genesis and progression of CLL, 80 cases were analyzed using G-banding and FISH with a panel of probes specific for 11q22.3(ATM), 12 centromere, 13q14.3, 13q34, and 17p13.1(p53). Genomic aberrations were detected by G-banding in 45% of the cases and by FISH in 65% of the cases. Single genomic aberrations were detected in 29 cases; 54% with deletion of 13q14.3, 25% with +12, 11% with deletion of ATM gene, 7% with del of p53, and 4% with deletion of 13q34. Among the 22 cases with multiple genomic aberrations, 84% with del(13)(q14.3), 47% with del(11)(q22.3), 37% with +12, 32% with del(13)(q34), and 21% with del(17)(p13.1). Correlations of the coexistence between individual genomic abberations were then inspected statistically. Trisomy-12 and deletion of 17p13.1 were found positively correlated with a p value of 0.003. Furthermore, a positive correlation at a lesser degree was found between deletion of 13q14.3 and deletion of 13q34 as well as between deletion of 13q14.3 and deletion of 11q22.3. Conversely, a negative correlation was found between the deletion of 13q14.3 and +12; deletion of 13q14.3 and deletion of 17p13.1; deletion of 11q22.3 and +12; and between 11q22.3 and deletion of 17p13.1. In summary, our data suggest that both del(13)(q13.4) and +12 are major primary genomic aberrations which occur independently in CLL onset. Then, +12 is followed frequently by deletion of 17p13.1 while deletion of 13q14.3 is followed frequently by deletion of 11q22.3 and deletion of 13q34. FISH is more efficient and sensitive in the detection of genomic aberrations than G-banding. However, G-banding can identify a broader scope of genomic aberrations, which are not included in the FISH panel and a combination of both approaches are crucial for CLL diagnosis.

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Detection of Alterations of Chromosome 17 And TP53 Gene In Solid Tumors By Dual- Color FISH. *J.C. Herrera-Patiño[1], L.F. Isaza-Jimenez[2], G. Vásquez[1], J.L. Ramírez-Castro[1], F. Quintero-Rivera[3], C.M. Muñetón-Peña[1].* 1) Medical Genetics Unit, Faculty of Medicine, Antioquia University, Medellín, Antioquía, Colombia; 2) Department of Surgery, Faculty of Medicine, Antioquia University, HUSVP, Medellin, Colombia; 3) Center for Human Genetic Research, MGH, Boston, MA.

TP53 is a tumor suppressor gene located on 17p13.1, this is essential for the control of cell cycle and has been found alterated in more than 50% of all tumors. In solid tumors, changes in the copy number of specific genes or chromosome and LOH are evaluated in interphase cell nuclei. Our aim was to examine alterations in primary solid tumors of chromosome 17 and TP53 gene. All samples were mechanically and enzimatically disaggregated with 0.2% collagenase. Dual-color FISH was performed using direct fluorescent labeling probes for the chromosome 17 centromere and TP53 gene in interphase nuclei from primary solid tumors of different origin. Hybridized signals were counted in 200 cells. 30 primary solid tumors of different origin were analyzed. Aneuploidy of chromosome 17 was found in 14 out of 30 tumors (47%). Monosomy was the most frequent aneuplody (57%); trisomy was detected in 2 cases (14%) and 4 tumors (29%) showed heterogeneous clones (monosomic, disomic, trisomic and tetrasomic). FISH analysis showed only 5 (16%) of 30 cases have 2 signals for the TP53 gene in more than 80% of the cells. One signal in more than 20% of the cells was observed in 22 cases (73%), whereas 1 case without signals and 2 cases with 3 signals were observed. In general we found in the most cases an imbalance of signals for chromosome 17 and TP53 per nucleus. LOH was observed in 7 out of 30 tumors (23%). All these cases showed an advanced stage in the development of cancer. In different solid tumors numerical alterations of chromosome 17 and TP53 gene deletions are common. Dual-color FISH detect these abnormalities per nucleus in individual cells of primary tumors, at this level we may have demonstrate that imbalance signals for chromosome 17 and TP53 gene and chromosome 17 aneuploidy had occurred during the development of cancer. The genetic instability of cancer cells is a common event which is caused by aneuploidy and gene mutation.

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A t(2;5)(p23;q35),+7 karyotype in a case of ALK+ anaplastic large cell lymphoma. *D. Wei[1], L.A. Cannizzaro[1], V.R. Pulijaal[1], M. Zohouri[1], D.T. Walsh[1], R.A. Conte[1], A.R. Deshikar[1], T. Zhou[1], C. Johnson[1], H. Ratech[1], E.A. Kolb[2], K.H. Ramesh[1].* 1) Dept Pathology, Montefiore Medical Ctr, Bronx, NY.10467; 2) Dept Pediatrics/Hematology/Oncology, Montefiore Medical Ctr, Bronx,NY, 10467.

Anaplastic large cell lymphoma (ALCL) has been recognized as a distinct clinicopathologic entity of T-cell or null cell lineage in the Revised European-American lymphoma classification. The t(2;5)(p23q35) in ALCL causes the fusion of NPM (nucleophosmin) gene located at 5q35 with the ALK (anaplastic lymphoma kinase) gene at 2p23. This gene fusion results in the generation of a novel chimeric protein: NPM-ALK, which has been shown to have oncogenic properties in a variety of experimental systems. The variant rearrangements in ALCL involving the ALK locus at 2p23 include: t(X;2)(q11;p23), t(1;2)(q25;p23), inv(2)(p23q35), t(2;3) (p23;q21), t(2;17)(p23;q23), t(2;19)(p23;p13.1) and t(2;22)(p23;q11.2). Expression of ALK gene in ALCL, either with t (2;5)(p23;q35), or other translocation variants, is known to be associated with a favorable prognosis. We report a 4-year-old female diagnosed with ALCL. The immunohistochemical stains revealed large atypical lymphoid cells that expressed CD3, CD30, CD45, ALK, AIA-1(T-cell intracellular antigen-1), granzyme B and a background population of scattered CD68 histiocytes. Flow cytometry showed a mixed T and B-cell population, without clonal B-cell expansion. Cytogenetic and FISH analysis of the lymph node revealed the karyotype: 47,XX,t(2;5)(p23;q35),+7[18]/46,XX[2] .ish t(2;5)(p23;q35)(wcp2+,wcp5+). nuc ish 2p23(pcp 2p sp). These results were consistent with the clinical diagnosis of anaplastic large cell lymphoma. Additional chromosomal abnormalities have been reported in about 20% of ALCL cases with t(2;5)(p23;q35). However, the significance of the secondary abnormalities is not clear. Studies with long-time follow up are essential in determining the significance of secondary chromosomal abnormalities associated with t(2;5)(p23;q35) in ALCL.

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A novel translocation (12;14)(q24.3;q11.2) in a patient with a therapy related AML. *Y. Maroz, S.Y. Dong, T.C. Brown.* Genzyme, Phoenix, AZ.

Cytogenetic abnormalities implicating the locus 12q24 are not rare in acute myeloid leukemia (AML). Several chromosomal partners involved in 12q24 translocations have been described in patients with AML such as 2q21, 3q21, 7q32, 14q32 and some others. We report a balanced translocation between 12q24.3 and 14q11.2 in a patient with a therapy-related AML. A 75-year-old male with a previous history of basal cell carcinoma was initially diagnosed with a myelodysplastic syndrome. At the time of the diagnosis he had leukopenia, thrombocytopenia and slight anemia. No abnormal cells were present by flow cytometry. The cytogenetic analysis on bone marrow revealed a karyotype of 46,XY,t(12;14)(q24.3;q11.2)in 16 of the 20 metaphases analyzed. Within two years the patient developed a relapse of AML. Flow cytometry analysis revealed 88% blasts, which coexpressed CD4, CD11, CD13, CD33 and CD38. This time the leukemic cells showed a karyotype of 46,XY,del(3)(q21q29),t(12;14)(q24.3;q11.2) in all 20 metaphases analyzed, which reflected an evolution of the original clone. The 14q11.2 breakpoint involved in this translocation is not typical for myeloid abnormalities. Two genes, T-cell Receptor Alpha (TRA) and T- cell Receptor Delta (TRD) have been well-described at locus 14q11.2. These genes are typically involved in translocations observed in T-cell acute lymphoblastic leukemia and lymphoproliferative disorders. According to data provided in the Atlas of Genetics and Cytogenetics in Oncology and Haematology there are approximately 17 other identified genes in the 14q11.2 region that may be involved in causing cancer. These include genes that play a role in DNA repair, cell death, and members of the RAS oncogene family. The gene(s) at 12q24 responsible for oncogenesis are not known at this time, however, there are several possibilities including genes involved in cell cycle regulation, transcription factors, and again members of the RAS oncogene family. Deletions of the long arm of chromosome 3 are a common finding in treatment-related myelodysplasia and AML. To our knowledge, this (12;14) translocation has not been reported previously in the literature and may aid in determining what genes in these two regions are involved in myeloid malignancies.

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A t(8;22)(q24;q11) In A Patient Diagnosed With a Rapidly Growing Plasmacytoma of the Scalp. *T. Mercado[1], A.L. Zaslav[1], K. Zamkoff[2], D. Gladstone[2].* 1) Cytogenetics, Suny Stony Brook, Stony Brook, NY; 2) Blood and Marrow Stem Cell Transplantation Program, University Medical Center, State University of New York at Stony Brook, N.Y.

A 49-year-old male presented with a rapidly enlarging scalp mass. Biopsy of the mass demonstrated by both morphology and flow cytometry a diagnosis of plasmacytoma. Radiographic bone survey, bone marrow and flow cytometry of the bone marrow aspirate showed no evidence of either multiple myeloma or lymphoma. Standard and molecular cytogenetic evaluation was performed on the bone marrow aspirate material. Cytogenetic results revealed a karyotype of 46,XY,t(8;22)(q24;q11),der(13)t(1;13)(q12;q22)[8]/46,XY[12]. FISH results on the original scalp mass using the 8q24 LSI MYC probe (Vysis, Downers Grove, IL) demonstrated a signal pattern consistent with an 8q24 translocation in 4 of 100 nuclei [i.e., nuc ish 8q24(MYCx1)[4]]. Based on the presence of the t(8;22)(q24;q11) in the bone marrow, the patient was diagnosed with multiple myeloma having an unusual karyotype i.e., t(8;22). Prior to knowledge of the bone marrow karyotype, the patient was treated with radiation therapy to the scalp mass. Three weeks later the patient returned with pain in the left hip. MRI showed evidence of new bone lesions, not present prior to this examination. Based on the rapid progression of the disease and the finding of the t(8;22) in the patients marrow and original scalp lesion, the patient was then started on an aggressive multiagent chemotherapy program used in high grade Burkitt like lymphomas. The only evidence of the systemic and aggressive nature of the patients disease originally was the presence of the t(8;22) found at the time of the original diagnosis. The patient is now in clinical remission and is awaiting an autologous stem cell transplant. This finding was significant in diagnosis, prognosis and treatment for this patient and will be useful in diagnosis of other patients with this disease.

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Dicentric (17;20)(p11.2;q11.2): An uncommon abnormality associated with poor prognosis in myeloid disorders.

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We report 2 patients with a dic(17;20)(p11.2;q11.2). The cytogenetic result of this dicentric chromosome is the partial loss of 17p and 20q. The dic(17;20) was confirmed by FISH. The first patient was a 74-year-old female with history of lymphoma since 1980, for which she received chemotherapy and radiation. Her recent blood findings included a WBC of approximately 3.5x103/L, a normal platelet count, a hematocrit of 30%, and a MCV of 103, suggestive of a myelodysplastic syndrome (MDS), likely therapy related. Chromosome analysis of her bone marrow showed an abnormal karyotype, which included a dic(17;20). The second patient was a 47-year-old male that presented with pancytopenia. His WBC was 6x103/L, hemoglobin was 6.0g/dL, and his platelet count was 20x103/L, without circulating blasts. His bone marrow was hypercellular (75% cellularity) with 58% blasts. Immunophenotyping showed that the blasts were of myeloid origin, expressing myeloperoxidase and the markers CD13, CD33, CD64, CD34, and CD117. The diagnosis of an acute myeloid leukemia (AML) was made at that time. Chromosome analysis of his bone marrow showed a complex karyotype, which included a dic(17;20). This patient subsequently received induction chemotherapy. At day 14, the marrow still showed persistence of the leukemia, for which the patient received additional rounds of chemotherapy. Deletions of 17p and 20q are recurrent abnormalities in various hematologic disorders including MDS and AML; however a dic(17;20) is an uncommon finding. According to the few reports in the literature, dic(17;20) is associated with an unfavorable prognosis. The prognostic outcome is similar to the one seen in the so-called 17p- syndrome. The common factor between the dic(17;20) and the 17p- syndrome is the loss of *TP53*, highlighting the critical role of this tumor suppressor gene in the development and/or progression of some hematologic disorders.

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ACUTE BASOPHILIC LEUKEMIA WITH TRISOMY 19 AND COMPLEX BCR-ABL. *A. Rojas-Atencio[1], M. Soto-Quintana[1], K. Urdaneta[1], F. Alvarez Nava[1], E. Avila[2], L. D`Salvo[3], J. Cañizalez[1].* 1) Unidad de Genetica Medica, Univ del Zulia, Zulia, Zulia, Venezuela; 2) Servicio de hematologia, Hospital Universitario, Maracaibo, Venezuela; 3) Instituto Hematologico de occidente Maracaibo, Venezuela.

Acute basophilic leukemia (ABL) has been recently included into the acute myeloid leukemias, proposed by the WHO panel. Due to the rarity of this disease diagnostic criteria are lacking. The objective of this report is to describe a case of ABL based upon of the clinical, morphological, cytogenetic and molecular evaluations. Patient was evaluated when she was 44 years old by widespread weakness, fever, and difficult to breath. Hepatospleen grade II was appreciated on phisycal examination. The haemoglobin was 5 g/dL, the white blood cell count was 23,1 x 109/L with 23 % of polymorphonuclear neutrophils, 20% of lymphocytes, 9% of monocytes, 2% of eosinophils, 2% of mielomonocytes, 4% of metamielocytes and 40% basophils. The thrombocyte count was 200,000/mL. The bone marrow was replaced by 80% of cells with basophil granules in their cytoplasm and 20% of immature nuclei. These cells were positive by black Sudan. Immunohistochemistry showed Dr=negative, CD34=12%, CD33=neg, CD14=neg, CD7=25%. Conventional cytogenetic analysis demonstrated a trisomy of the chromosome 19 and a translocation 9; 22 (Ph chromosome +). FISH analysis showed the presence of the complex BCR-ABL in 95% of the screened nuclei. In the patient ABL was diagnosed on these findings. The patient was treated with hidroxiurea which resulted in partial remission. The patient died 2 months of the initial diagnosis. The clinical and morphological features and the dramatic evolution in this patient make us suspect in ABL with Philadelphia chromosome positive. A better definition of the diagnostic criteria is necessary for this illness.

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Constitutional Partial 1q Trisomy Mosaicism and Wilms Tumor. *H.F.L. Mark[1,2], H. Wyandt[1,2], A. Pan[1], J.M. Milunsky[1,3,4].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pathology,Boston University School of Medicine, Boston, MA; 3) Department of Pediatrics,Boston University School of Medicine, Boston, MA; 4) Department of Genetics and Genomics,Boston University School of Medicine, Boston, MA.

We report a female patient with severe-profound mental retardation, multiple congenital anomalies, and a history of mosaicism for partial 1q trisomy in amniotic fluid and a previous Wilms tumor specimen. Peripheral blood and fibroblasts were studied and did not demonstrate the mosaicism initially detected for chromosome 1q. Array comparative genomic hybridization (CGH), yielded negative results. Additional cytogenetic studies were performed that help clarify the previous findings, and reveal evidence of partial 1q trisomy mosaicism in normal kidney tissue and in a kidney lesion. GTG-banded results showing low-percentage mosaicism for the structural rearrangement der(1)t(1;1)(p36.1;q23) in both tissues are corroborated by fluorescent in situ hybridization (FISH) studies. We hypothesize that the partial 1q trisomy predisposed the target tissue (in this case kidney) to neoplasia. This study provides further support for the hypothesis that certain constitutional chromosomal abnormalities can predispose to cancer. As detection of a low percentage mosaicism may be hampered by the limits imposed by currently available technology and the constraint of a finite sample size, extra vigilance in monitoring other somatic tissues will be needed throughout the patients lifetime. Anticipatory clinical guidance and prognostication are meaningful only if given accurate cytogenetic diagnoses. To the best of our knowledge, this is the first reported case of Wilms tumor associated with constitutional partial 1q trisomy, either in pure or mosaic form, or with the particular 1q23 breakpoint in conjunction with a break on 1p36.1.

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Malignant transformation of 45,X and 46,XY cell lines in gonadoblastoma arising in dysgenetic gonads. *A. Al Saadi.* Dept Anatomic Pathology, William Beaumont Hosp, Royal Oak, MI.

TITLE: Malignant transformation of 45,X and 46,XY cell lines in gonadoblastoma arising in dysgenetic gonads Gonadoblastoma (GB) develops in 15-20% of 45,X/46,XY individuals. GBY gene on the Yq is believed to predispose to GB. It is not known however if both 45,X and 46,XY cell are equally involved in the developing GB. In this report we present cytogenetic and FISH of bilateral GB in a 15 1/2-year-old, phenotypic female with hypogonadism. The history includes breast development at age 11, normal growth, normal pubic hair and 3 menses at age 13 followed by amenorrhea. Lab results revealed elevated FSH and LH but normal TSH, T4, prolactin and estradiol. Blood chromosomal analysis revealed a 46,XY karyotype. Pelvic ultrasound showed normal uterus and a left gonad. MRI revealed a small atrophic right gonad. Echocardiogram was normal with no coarctation of the aorta. Bilateral GB was diagnosed following gonadectomy. Tissue from both sides was submitted for cytogenetic analysis. Fifty spreads from each side were analyzed. The left had 54% with 45,X and 46% with 46,XY complements. The right had 76% 45,X and 24% of 46,XY complements. Tissue sections, from various parts of both sides, which had different histopathology, were hybridized with CEPX and CEPY probes. Two hundred nuclei from each of DA, sex chord element (SC) and stromal tissue (ST) were scored. All three tissues had 45,X/46,XY constitution. In the DA and SC 69% of the cells had X and Y signals and 31% had X signals only. In the ST, 41% had X and Y signals and 59% had X signals only. These results suggest that both 45,X and 46,XY cells undergo transformation to form GB. Further studies are needed to determine if either the 45,X or 46,XY cells are more susceptible to form GB.

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A novel t(14;20)(q32;p13) in acute myelogenous leukemia. *M.P. Ornelas, C.S. Berger, S. Karamanov, T.C. Brown.*
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A bone marrow sample from a sixty-two year old male was evaluated for a clinical diagnosis of leukemia. Flow cytometry results were suggestive of acute myelomonocytic leukemia and cytogenetic results were normal 46,XY. A month later, flow cytometry results were consistent with a remission bone marrow. Follow-up cytogenetic testing three months after the initial cytogenetic study showed 7 of the 20 cells analyzed with the following abnormal karyotype: 46,XY,add(14)(q32),del(20)(q11.2q13.3). Cytogenetic studies performed five months later showed 13 of the 20 cells analyzed to have the same abnormal karyotype. Six months later all 20 cells were abnormal. With much better resolution on this final sample, it was questionable whether the deletion of chromosome was actually 20q or possibly 20p. FISH probes for the 20q12 region revealed two signals thereby showing the deletion was on 20p not 20q as was reported. Hybridization with the IGH probe at 14q32.3 showed that this gene was not directly involved but was translocated to the der(20). Whole chromosome paints of chromosomes 14 and 20 provided final evidence that the actual karyotype was a balanced translocation between 14 and 20, 46,XY,t(14;20)(q32;p13). This is quite unusual since 20q is typically involved in myeloid disorders. This is a new, previously unreported translocation associated with acute myelogenous leukemia.

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Amplification of the MLL gene localized by FISH on dmins and hsrs in a patient with RAEB-2. *C. Sreekantaiah, P.K. Berry.* Dianon Systems/Labcorp, Stratford, CT.

Amplification of the MLL gene is a rare but recurrent aberration in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). It is associated with karyotypic complexity and adverse prognosis. We report the cytogenetic and fluorescence in-situ hybridization findings in an 81-year-old woman with refractory anemia with excess blasts (RAEB-2). The patient received palliative care and died shortly after diagnosis.

Cytogenetic analysis of the bone marrow revealed two related clones with multiple and complex chromosomal abnormalities, including a ring chromosome, double minute chromosomes (dmins) and homogeneously staining region (hsr), the latter two representing cytogenetic manifestations of gene amplification. The karyotype was as follows: 44~46,XX,der(9)ins(9;11)(q11;q11q23),-11,-17,der(20) t(17;20)(q11.2;q11.2),+0-1r,0~11dmin [cp6]/44~46,XX,der(9)ins(9;11)(q11;q11q23)inv(11)(q13q21)hsr(11)(q23), -11,-17,der(20)t(17;20)(q11.2;q11.2),+0-1r[cp14].

FISH analysis with the MLL probe (Vysis, Inc.) showed multiple hybridization signals on the ring chromosome, the dmins and within the hsr at 11q23 reflecting amplification of the MLL gene.

Our findings are unique in that both intrachromosomal and extrachromosomal amplification of the MLL gene were noted in the same patient. It also confirms the poor prognostic outcome associated with MLL amplification and karyotype complexity.

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A rare but recurrent chromosome abnormality, ider(9)(q10)t(9;22)(q34;q11.2) identified in a patient with ALL.

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A t(9;22)(q34;q11.2) resulting in a chimeric BCR-ABL gene is found in 95% of patients with chronic myeloid leukemia (CML), 5% of children with Acute lymphoblastic leukemia (ALL) and 20% of adults with ALL. The chimeric BCR-ABL gene on the derivative 22 chromosome encodes a fusion protein with constitutive protein kinase activity contributing to malignant transformation and growth. Rearrangements of the der(22) and der(9) chromosomes generated by the t(9;22) as well as acquisition of other chromosome anomalies have been documented as part of disease progression in hematologic disorders with a t(9;22)(q34;q11.2). Rearrangements of the der(22) are observed more often, whereas changes to the der(9) are rarer. Here we present a case, a 23-year-old female with ALL associated with ider(9)(q10)t(9;22)(q34;q11.2). Fluorescence in situ hybridization (FISH) studies on bone marrow using the dual-fusion dual-color FISH probe for BCR and ABL showed three fusion signals suggestive of an extra copy of der(22) or der(9), and the karyotype obtained from G-banded chromosome analysis was 46,X,der(X)t(X;1)(q28;q11),ider(9)(q10)t(9;22)(q34;q11.2),der(22)t(9;22)(q34;q11.2)[3]/46,XX[17] leading to a net gain of 1q, 9q and proximal 22q as well as loss of 9p. Reverse transcriptase polymerase chain reaction (RT-PCR) on peripheral blood detected a BCR-ABL gene fusion transcript coding for a 190 kd protein. Flow cytometry results and bone marrow morphology were consistent with precursor B lymphoblastic leukemia/lymphoblastic lymphoma. So far six cases of ider(9)(q10)t(9;22)(q34;q11.2) have been reported, including three individuals with lymphatic blastic phase of CML and 3 patients with ALL. Survival data have been documented in only 4 of them. The present case coupled with published cases show that development of an ider(9)(q10)t(9;22)(q34;q11.2), though seemingly rare, is a pathway of progression in hematologic disorders with a t(9;22)(q34;q11.2).

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MYC Amplification in Acute Myeloid Leukemia. *S. Sait[1], L.A. Ford[3], M. Barcos[2], M.R. Baer[3].* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Ctr, Buffalo, NY; 2) Department of Pathology, Roswell Park Cancer Ctr, Buffalo, NY; 3) Department of Medicine, Roswell Park Cancer Ctr, Buffalo, NY.

Although occurring frequently in solid tumors, gene amplification is reported in only ~ 1% of karyotypically abnormal leukemias, with double minute chromosomes (dmin) and homogenously staining regions (hsr) as its cytogenetic hallmarks. Regions commonly amplified in leukemias include 8q24 (MYC) and 11q23 (MLL). We report 7 patients with acute myeloid leukemia (AML) seen at Roswell Park Cancer Institute since 1985 with dmin in whom amplification of the MYC gene was demonstrated by fluorescence in situ hybridization. Six patients were elderly (>75 years) and six were females. FAB types were diverse. Two patients had therapy-related AML, and one had a prior myelodysplastic syndrome. Five had other chromosome abnormalities in addition to dmin. Six patients had primary refractory disease, and one had only a brief remission. Thus MYC amplification is rare in AML, and is associated with a poor response to treatment. The mechanism of generation of double minutes is incompletely understood, although it has been suggested to occur through extra replication (or loop- formation)-excision-amplification. Studies have also shown that the amplicon generated contains other genes that might be functionally important, and thus MYC may not be the only target gene.

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Chromosomal Instability, Centrosome Aberrations and Expression Changes in BRCA1-pathway dependent Genes in Sporadic Ovarian Carcinoma. *J. Bayani[1, 2], M. Yoshimoto[1], B. Vukovic[1, 3], J. Weberpals[1, 2], M. Zajac[1], P. Marrano[1], J. Karaskova[1], B. Rosen[2], M. Zielenska[4], J.A. Squire[1, 2, 3].* 1) Dept.Cell&Mol Bio, Ontario Cancer Institute; 2) Dept.Lab. Med.& Pathobio; 3) Dept.Med. Biophys. University of Toronto; 4) Dept.Lab Med& Pathobio. Hosp.for Sick Children, Toronto.ON.

Ovarian cancer is the leading cause of death from a gynecological malignancy among North American women. Cytogenetic analysis shows them to be highly aneuploid with complex chromosomal aberrations suggesting errors in DNA repair and cytokinesis. Since somatic mutations of BRCA1 are rare in sporadic disease, it has been postulated that they play a limited role in its pathogenesis. However, the inactivation of BRCA1 at the RNA is relatively frequent in sporadic cases, suggesting a shared feature of between BRCA-mutated and non-BRCA-mutated ovarian tumors is the dysfunction of BRCA-dependant pathways, influencing the observed karyotypic and genomic complexity. Chromosome instability (CIN) studies were performed for 11 sporadic ovarian cancer specimens, from 8 patients previously described by our group (Bayani et al 2001) using SKY, CGH and interphase FISH. Centrosome amplification was also determined by immunostaining. In 5 patients, microarray analysis using the GEArray-Q DNA Damage Array was performed. Increasing CIN was associated with increasing centrosome aberrations. Diploid/near-diploid tumors were characterized by low-level CIN and centrosomal aberrations, but high frequencies of chromosomal breakage. Tetraploid tumors showed increasing CIN and centrosomal aberrations, with triploid tumors having the greatest CIN and centrosomal aberrations. In all tumors studied, microarray findings revealed reduced expression of BRCA1 to be statistically significant (p-value 0.01). Reduced expression of APAF1, ATM, PPMID, PRKDC and RAD50 was also detected in all tumors. Overexpression of HIST1H2AC and KIF23 was identified in all tumors tested. These findings suggest that impaired DNA repair and cell cycle checkpoints along BRCA1 pathways occur in sporadic ovarian cancers and play a contributing role in the pathogenesis of the disease.

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Loss of 17p is a major consequence of whole-arm chromosome translocations in hematologic malignancies. *A. Adeyinka[1], D. Kramer[1], D. Van Dyke[1,2].* 1) Dept Medical Genetics, Henry Ford Health System, Detroit, MI; 2) Dept Lab Medicine and Pathology, Mayo Clinic, Rochester, MN.

Whole-arm translocations reportedly are rare in hematologic malignancies. Indeed, very few whole-arm translocations have been documented as sole anomalies in hematologic malignancies. In order to ascertain the distribution of whole-arm translocations and their consequential imbalances, we surveyed the cytogenetic database at the department of Medical Genetics, Henry Ford Health System. Imbalances related to chromosomes involved in clonal acquired whole-arm translocations were ascertained from tumors with near-diploid karyotypes. One hundred and one (101) tumors met the search criteria and seven of these had balanced whole-arm translocations. The remaining 94 samples had whole-arm translocations that rarely or never involved chromosome 2, 3, 4, 6, 19, 20 or the sex chromosomes. Chromosome arms were lost more often than they were gained and p-arms were lost more than q-arms except for chromosome 7 (17% q-arm loss and 2% p-arm loss) and chromosome 11 (both arms were equally lost). Chromosome 1 q-arm was the only chromosome arm substantially gained (30% of tumors). 7p and acrocentric long arms were involved in 50% and 25%, respectively, of whole-arm translocations resulting in gain of 1q. Acrocentric chromosome were involved in acquired whole-arm translocations at a frequency of 4% (G-group) and 12-14% (D group), and were more likely to be involved in non-Robertsonian than Robertsonian translocations (P= 0.0027, Normal test). Loss of 17p was the most common p-arm loss, was present in 16% of tumors (myeloid and lymphoid), and often occurred as part of complex karyotypes suggestive of disease progression. Deletion of the TP53 gene is often regarded as an important molecular genetic outcome of loss of 17p. The present findings show that acquired whole-arm chromosome translocations in hematologic malignancies are nonrandom and that loss of 17p, often associated with a poor prognosis in a wide spectrum of hematologic malignancies as well as with the 17p- syndrome, is an important consequence of whole-arm translocations in these disorders.

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Genome-wide SNP scan detects submicroscopic chromosomal aberrations in acute lymphoblastic leukemia. *L-H. Li[1], A. Yu[2], Y-C. Lin[2], M. Dicciannni[2], S-F. Ho[1], C-H. Chen[1], K-C. Jiang[1], J-Y. Wu[1], Y-T. Chen[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Genomics Research Center, Academia Sinica, Taipei, Taiwan.

Cytogenetic analysis of acute lymphoblastic leukemia (ALL) is difficult due to the low mitotic rate and poor quality of the metaphases; the reported chromosomal abnormalities are less frequently observed in T-ALL than in B-ALL. Here we report the ultilization of Affymetrix GeneChip Human Mapping 100K set to detect both submicroscopic chromosomal abnormalities and allelic alterations in ALL patients (5 T-ALL and 1 B-ALL). Using this technology, we identified chromosomal aberrations in all but one T-ALL samples. One patient had double trisomy for chromosomes 18 and 21. Deletion of 6q, a frequent structural abnormality in ALL, was detected in another patient. Most significantly, we detected six loci with deletion less than 4.0 Mb in five samples. A deletion interval of 1 Mb within 14q11.2, where the TRAC gene is located, was identified in two samples. This suggests that deletion involving 14q11 is not infrequent in ALL patients. The lower rate of previously reported cases is mot likely due to the limited resolution of detection methods. We also identified deletion involving 13q14 in two samples. Interestingly, the deletion interval was 1.3 Mb within 13q14.2 including the RB1 gene in one sample, and 3.6 Mb within 13q13.3 to 13q14.11 in the other sample. In addition to samll deletions, one sample showed LOH of 9p without any sign of chromosomal copy number change, suggesting the existence of uniparental disomy. Taken together, these results demonstrate the power of the genome-wide SNP analysis in detecting small deletions and allelic alterations, and in defining the boundaries of cytogenetic abnormalities, which is useful in the seletion of candidate genes for further study to increase our understanding of leukaemogenesis.

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Development of a robust algorithm for genetic alterations in cancer genomes using Affymetrix SNP-genotyping microarrays with its applications to large-scale copy number/LOH/allelic imbalance mapping of cancer genomes.

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Genetic alterations are cardinal features of cancer genomes and inseparably related to the pathogenesis of cancers. Since cancer cells may heavily accumulate large number of genetic changes, genome-wide detection of these changes using high-throughput techniques is essential clue to the comprehensive understanding of how cancer develops. Here we report a robust algorithm for detecting genetic alterations of cancer genomes using Affymetrix GeneChip mapping arrays, which were originally developed for large-scale SNP genotyping in genetic epidemiologic studies. Advantages of this algorithm include the increased signal-to-noise (S/N) ratios and the use of optimized references. The raw S/N ratios were improved by accounting for the length and GC content of the PCR product using quadratic regressions. The use of a self-reference, when available, gives the lowest standard deviation (SD) values and also allows allele-specific copy number detection in cancer genomes, which can unmask otherwise concealed allelic imbalances. In the absence of a self-reference, optimized selection of multiple best-fit references dramatically improves SD values. These improvements allow for highly reliable comparison of data across different experimental conditions, detection of allele-based copy number changes, and more accurate estimations of the size of copy number aberrations. Using this algorithm we analyzed more than 300 tumor samples and thereby generated large-scale copy number/LOH/allelic imbalance maps, which unveiled a number of common regions harboring genetic abnormalities in cancer genomes and also underpinned that the Affymetrix 100K or 500K mapping arrays, in combination with the newly developed algorithm (CNAG; Copy Number Analyzer for Affymetrix GeneChip), provide an ideal platform for finely analyzing cancer genomes having complex abnormalities.

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Variant chronic myelogenous leukemia (CML) patients: a clinical and molecular cytogenetic study. *A. Block[1], S.N.J. Sait[1], S. Kakati[1], P. Starostik[2], M. Wetzler[3].* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Molecular Diagnostics Lab, Roswell Park Cancer Inst, Buffalo, NY; 3) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

Chronic myelogenous leukemia (CML) is a clonal hematologic malignancy that originates in an abnormal pluripotent bone marrow stem cell. The majority of patients (pts) have the characteristic t(9;22)(q34;q11.2) which fuses sequences of the BCR gene with the ABL gene. The clinical course of CML is heterogeneous, with inter-individual variability in clinical and laboratory findings at diagnosis, in the rate of progression to blast crisis (BC), and in survival. The nature of this heterogeneity and the molecular basis of the progression to BC are poorly understood. To determine the effect of baseline cytogenetic characteristics on the efficacy of current drug (imatinib) therapies, we identified a subgroup of 12 CML pts treated at our institution within the past three years with normal karyotypes (NK), complex variant translocations (CVT), and/or atypical BCR/ABL fluorescence in situ hybridization (FISH) patterns often resulting in microdeletion events. Molecular responses to therapy were seen at 3 months in 1/2 pts with NK, 1/2 evaluable pts with CVT and 5/5 evaluable pts with derivative chromosome 9 deletions (der9del). Complete responses (CR) to therapy were observed in 1/2 pts with NK and 3/7 pts with der9del. Hematologic and/or cytogenetic responses were achieved in 2/7 pts with der9del and 1/1 evaluable pts with CVT. Disease progression occurred in 1/3 der9del pts with CR. Longer follow-up and study of more pts will be required to determine the effects of microdeletions on disease progression and overall survival. Time-line of patient response and details of molecular cytogenetic abnormalities will be presented.

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Molecular Cytogenetic Basis of 2+ HER-2/neu IHC Expression in Breast Cancers: A FISH Study of 110 Cases.
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Purpose: Proto-oncogene HER-2/neu is an important biomarker in breast cancer and its amplification is correlated with poor prognosis. Specific treatment protocols are available to breast cancer patients with amplified HER-2/neu gene. HER-2/neu status is currently assessed by immuno-histochemistry (IHC) as a primary diagnostic test and 2+ IHC cases are reflexed to HER-2/neu testing by FISH to determine the amplification status of the gene. A significant number of cases that are 2+ positive for HER-2/neu by IHC are found to be discordant with the FISH results. We investigated the underlying molecular cytogenetic abnormalities of breast cancer cases with 2+ HER-2/neu IHC expression by FISH. Methods: 110 cases of breast carcinoma with 2+ IHC HER-2/neu expressions with both IHC and FISH HER-2/neu status available were selected after IRB approval. IHC and FISH were performed at Women & Infants Hospital using FDA approved Dako Cytomation reagent for IHC (HercepTest) and VYSIS (PathVysion) detection system for FISH. IHC was scored according to the manufacturers guidelines as 0-1+ (negative), 2+ positive and 3+ positive. FISH results are interpreted as HER-2/neu signal over chromosome 17 signal ratio of <1.8 as non-amplified, >2.2 as amplified and 1.8-2.2 as borderline amplified. Results: 31 of 110 (27%) cases had underlying molecular cytogenetic abnormalities detected by FISH under one of four categories: 8 cases were HER-2/neu amplified; 3 borderline amplified; 11 with focal amplification (few cells were amplified but not significant to alter the ratio); and 9 cases with polysomy of chromosome 17. Conclusion: A significant number (27%) of breast cancer with 2+ HER-2/neu IHC expression showed underlying molecular cytogenetic abnormalities detected by FISH. Two categories, borderline and focal HER-2/neu amplification, may be of clinical significance and possibly represent the earliest phase of HER-2/neu amplification. It may be prudent to treat these subsets of breast cancers similar to bona fide HER-2/neu amplified cases.

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Complex t(der(8)t(8;14);18)in an aggressive follicular lymphoma. *A. Meloni-Ehrig[1], C.A. Tirado[1], J.C. Kelly[1], C. Caldwell[1], J. Jahn[1], J. Scheerle[1], P. Beveridge[2], W.M. Ueno[2], P.. Francis[2], C.D. Croft[1], D. Heritage[1], P.N. Mowrey[1].*
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We report a rare complex t(8;14;18)(q24;q32;q21) involving simultaneously the *C-MYC(8q24)*, *BCL2 (18q21)*, and *IGH (14q32)* regions, which was confirmed by FISH. The patient is a 63-year-old male diagnosed with low-grade non-Hodgkin lymphoma (NHL) in 2002 for which he received chemotherapy but failed to achieve complete remission. Flow cytometry performed in February 2005, detected a monoclonal CD10+ population. CD10 can be seen in follicular lymphoma (FL), Burkitt lymphoma (BL), immunoblastic lymphoma, and diffuse large B-cell lymphoma (DLBCL). Chromosome analysis of a recent bone marrow aspirate showed a complex karyotype with numerical and structural abnormalities including a three-way t(8;14;18) in 12/20 metaphases analyzed. A similar variant t(8;14;18)(q24;q32;q21) has been reported in few cases of NHL and acute lymphoblastic leukemia (ALL). Commonly, t(8;14)(q24;q32) is recurrent in ALL and NHL, especially BL, whereas t(14;18)(q32;q21) is typically seen in 85% of FL and 20-30% of DLBCL. The concurrent FISH study was performed using the dual-color dual-fusion translocation probes *IGH/MYC/CEP8* and *IGH/BCL2* (Vysis, Downers Grove, IL). The probe IGH/MYC/CEP8 detected a variant pattern: two *IGH* signals were located on the two derivative chromosomes 18 and two *IGH/MYC* fusion signals were located on the derivative chromosomes 8 and 14. The *IGH/BCL2* probe showed a variant pattern, which consisted of three *IGH/BCL2* fusion signals: two on the derivative chromosomes 18 (*BCL2* region) and one on the derivative chromosome 8 (*C-MYC* region). As a result, the derivative chromosome 8 contained both the *IGH/MYC* and the *IGH/BCL2* fusion signals, suggesting a possible 2-step mechanism in the generation of the t(8;14;18). These findings, i.e., the simultaneous activation of *C-MYC* and *BCL2* by translocation with the IGH locus, are suggestive of an aggressive type of FL.

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Tumor Genome Anatomy Project (TGAP); Gene discovery in tumors in the genome era. *S.D.P. Moore[2, 4], O. Offor[1], J. Feryi[3], P. Amrein[3], P. Dal Cin[1, 4], C.C. Morton[1,2,4].* 1) Dept Pathology, Brigham & Women's Hosp., Boston, MA; 2) Dept of Obstetrics, Gynecology and Reproductive Biology, Brigam & Women's Hosp., Boston, MA; 3) Hematology-Oncology Unit, Massachusetts General Hosp., Boston, MA; 4) Harvard Medical School, Boston, MA.

TGAP is a new initiative to identify genes involved in neoplasia in a high throughput manner taking advantage of naturally occurring human genomic and genetic changes resulting from chromosome rearrangements in individuals with a wide spectrum of neoplasms. The completion of the Human Genome Project is redefining the rapidity of genetic discoveries with potential clinical applications. TGAP will exploit these growing biological resources, and will coordinate an integrated group of researchers with expertise in cytogenetics, molecular biology and oncology. This gene discovery initiative is a natural extension in the application and translation of the complete human DNA sequence.

The current strategy of TGAP is first to identify a tumor with novel rearrangements primarily from tumor samples submitted for analysis to the Cytogenetics Laboratory at the Brigham & Women's Hospital. Chromosomal breakpoints are mapped by FISH to discover candidate genes or regions of conserved non-genic sequence. Molecular analysis ensues to pinpoint potential disruption or position effects on gene function.

We have uncovered two novel fusion transcripts associated with Acute Myeloid Leukemia. Preliminary work on a t(8;9)(p22;p21.3) rearrangement indicates a fusion transcript between *ASAH1* and *MLLT3*. ASAH1 has a role in Sphingolipid metabolism and associated with Farber disease but has not previously been shown to be involved with cancer. The second rearrangement involves a t(X;21)(q26;q22). In this case, a fusion transcript between *ELF4* and *ERG* is created. This novel fusion is between two known *ETS* family members and is the first evidence that directly supports a role for *ELF4* in cancer.

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Multicolor fluorescence in situ hybridization (SKY) in mycosis fungoides and Sezary syndrome: search for recurrent chromosome abnormalities. *D.A.S. Batista[1], A. Hawkins[1], L. Morsberger[1], P. Long[1], E. Vonderheid[2], C.A. Griffin[1].* 1) Dept. of Pathology; 2) Dept. of Dermatology, Johns Hopkins University, Baltimore, MD.

Cutaneous T-cell lymphomas are clonally derived lymphoproliferative disorders that preferentially involve the skin. The two major clinical expressions, mycosis fungoides (MF) and Sezary syndrome (SS) may develop overt lymph node and/or blood involvement during the course of the disease. Pathogenesis is poorly understood for either entity. Chromosome abnormalities, mostly complex karyotypes are seen in about 50% of patients with MF/SS and there are only few instances of recurrent rearrangements. We analyzed 19 blood samples from patients with MF/SS with conventional cytogenetics and SKY to better describe the complex karyotypes and search for recurrent abnormalities or breakpoints. Comparison of phytohemagglutinin (PHA) versus a combination of interleukin 2 plus interleukin 7 showed similar efficiency to detect abnormal clones for both mitogens, however the PHA cultures yielded more analyzable metaphases. 9/19 patients (47%) had an abnormal karyotype. Most frequent abnormalities involved chromosome 10 in 7/9 cases, followed by chromosome 6 in 6/9, chromosomes 3, 7, 9, 17 and 19 in 5/9, chromosomes 1 and 12 in 4/9 and chromosomes 8, 11, and 13 in 3/9. Most abnormalities were structural. Recurrent rearrangements included deleted chromosomes 6 and 13 in three cases each and recurrent breakpoints were 1p32-36, 6q22-25, 17p11.2-13, 10q23-26 and 19p13.3 occurring in three or more cases each. One patient had a pseudo dicentric translocation between the short arms of chromosomes 8 and 17, confirmed by dual color FISH and interpreted as psu dic(17;8)(p11.2;p11.2). Two patients with SS reported by Thangavelu et al.(Blood 1997, 89: 3371) had a similar translocation described as der(8)t(8;17)(p11;q11). Molecular cytogenetics was not performed in these two cases. It seems plausible based on the pictures published that these three cases represent the same rearrangement. If indeed this is true, a psu dic(17;8) is the first recurring translocation detected in a set of three patients with MF/SS.

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Atypical D-FISH pattern in a patient with Juvenile Myelomonocytic Leukaemia: A case of positive Philadelphia chromosome? *G.C. Ramírez-Gaviria[1], C.M. Muñetón-Peña[1], F. Cuellar-Ambrossi[2], M. Sarmiento[2], F. Quintero-Rivera[3], J.L. Ramírez-Castro[1], G. Vásquez-Palacio[1].* 1) Medical Genetics Unit, Antioquia University, Medellín, Antioquía, Colombia; 2) Department of Hematology, Faculty of Medicine, Antioquia University, HUSVP, Medellin, Colombia; 3) Center for Human Genetic Research, MGH, Boston, MA.

A 16 months old male with fever and bronchitis was seen in June 2004. The spleen and liver were palpable 5 cm below the rib margins. Differential counts showed WBC 73.000 mm3 , myeloid hyperplasia with monocytes 11.6%, CD34 1.9% and high levels of IgM for cytomegalovirus. A hepatic biopsy suggests a Juvenile Myelomonocytic Leukaemia (JMML). Cytogenetic analysis with RGH banding on a peripheral blood specimen revealed a male normal karyotype 46,XY in 40 metaphases. D-FISH analysis for BCR/ABL was performed using a dual colour dual fusion spectrum orange/green probe set (Vysis). Hybridization with this probe in typical BCR/ABL rearrangement produce two fusion signals (yellow F) corresponding to BCR/ABL fusion on a der(22) chromosome and ABL/BCR fusion on a der(9) chromosome, in addition to one green signal and one orange signal on both normal 9 and 22 chromosomes respectively. In this patient, the hybridization pattern was normal in 90% of the interphase nuclei (90) (2O2G) corresponding to negative Philadelphia chromosome, however 10% of the cells (10 nuclei) showed atypical fusion patterns with loss of one fusion signal: 1O1G1F, 1O2G, 2O1G1F. The single fusion pattern was detected by interphase D-FISH but not in metaphase cytogenetics analysis and may represent either BCR/ABL or ABL/BCR gene fusion. It has recently been shown that the atypical D-FISH patterns may be caused by microdeletions in association with the breakpoints or DNA loss around the breakpoint in 9q34. These atypical fusion patterns are present in near 20% of patients with Chronic Myeloid Leukaemia and they must be observable in above of 1.8% of analysed cells. Those rearrangements have not been reported in JMML. Additional studies are in progress to further syndrome delineation and the molecular characterization of prognosis factors in this patient.

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Identifying and mapping the human penumbra gene to a region on chromosome 7 frequently deleted in myeloid malignancies. *Z. Chen[1], M. Pasquini[2], B. Hong[1], S. DeHart[2], M. Heikens[2], S. Tsai[2].* 1) Dept Pediatrics/Cytogenetics, Univ Utah Sch Medicine, Salt Lake City, UT; 2) Division of Hematology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah.

In a search for new genes that might be involved in the regulation of hematopoiesis, we performed cDNA representational difference analysis on the multipotent murine hematopoietic cell line, EML C1 , using a syngeneic promyelocytic cell line, MPRO, as the subtractor. One of the new genes that we have identified is the murine Penumbra (for proerythroblast nu[new] membrane), which exhibits differential expression in proerythroblasts/erythroblasts in Northern analysis. Penumbra encodes a new member of the evolutionarily conserved tetraspanin membrane protein family that includes CD9, CD53, CD63, CD81, CD82, CD151 and peripherin. Using murine Penumbra as a probe, we identified human Penumbra from a normal human bone marrow cDNA library. The human Penumbra encodes a protein of 283 amino acids and is 97% identical to the murine Penumbra protein. This high degree of sequence homology suggests that Penumbra serves an important function. Interestingly, an expressed sequence tag (est AI480218) corresponding to the end of the 3 untranslated region of human Penumbra cDNA is predicted to be at chromosome7q32.1 in the National Center for Biotechnology Information (NCBI) human genome database. This region of chromosome 7 is a hotspot for cytogenetic abnormalities in myeloid malignancies associated with poor prognoses. Based on these data, we further designed a human Penumbra probe contig and used fluorescent in situ hybridization (FISH) to analyze seven cases of myeloid malignancies with 7q deletions. Five patients with cytogenetic deletions involving 7q31.2q32 also showed deletions of Penumbra by FISH; these were not present in two patients with cytogenetic deletions not involving 7q31.2q32. Our findings provide the first FISH evidence supporting the mapping of human Penumbra to 7q31.2q32 and demonstrate the potential use of the Penumbra probe in the detection of 7q31q32-related deletions in myeloid malignancies.

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High resolution analysis of linearized cancer genomic DNA using parallel nanochannel arrays. *H. Cao, P. Deshpande, M. Austin, C. Tan, M. Boyce-Jacino.* BioNanomatrix, Inc. 3701 Market St, Suite 340 Philadelphia, PA 19104.

We are developing a nanochip device for manipulating long genomic DNA for high-resolution (< kilobase), whole-genome analysis of cancer biomarkers such as gene amplifications, deletions, and translocations. These chromosome structural aberrations are strongly implicated in the process of malignant transformation,and are important diagnostic, prognostic, and therapeutic indicators for many types of cancer. Techniques that rely on probing chromosomes, such as metaphase FISH, while providing a pan-genomic view, cannot resolve structures below the Mb range. By probing uncompressed interphase DNA, resolution can be improved, but spatial organization of the genome is lost, so multiplexed and quantitative information is difficult to obtain. By stretching out (linearizing) interphase DNA, using techniques such as molecular combing or optical mapping, it is possible to probe specific loci in a spatially-significant way, and with resolutions in the kb range. However, techniques for mechanically linearizing DNA are inherently variable, leading to inconsistent stretching of molecules, which often cross over and retract upon themselves. We are developing an innovative alternative to mechanical stretching of DNA. We have found that megabase individual DNA molecules because of the self-avoiding nature of the DNA polymer, will elongate and straighten in a consistent manner when streamed into confining nanometer-scale channels (nanochannels)in massive parallel fashion (50,000 channels on 1 cm nanochip). We have used a novel nanofabrication technique to manufacture nanochannel structures in silicon chips, and have demonstrated that linear DNA in these nanochannels can be measured with precision of < 400 bp within 1 minute. We are quantitatively interrogating these linearized DNA with locus-specific probes for the detection of genomic instability events associated with cancer. Our product, the nanochannel array chip, will comprise part of an integrated platform for the routine and standardized quantitative analysis of cancer DNA structure that will enable archiving and cross-laboratory comparison of data.

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Complex rearrangement of *cMYC, BCL2, BCL6,* and *IGH* in an aggressive B-cell lymphoblastic lymphoma/leukemia. *C.W. Rehder[1], P.J. Buckley[1], J.Z. Gong[1], A.S. Lagoo[1], J.O. Moore[2], B.K. Goodman[1].* 1) Dept. of Pathology; 2) Dept. of Medical Oncology & Transplantation, Duke University, Durham, NC.

Concurrent translocations involving *cMYC/IGH* as well as *BCL2* or *BCL6/IGH* have been reported previously in cases of aggressive lymphoma. We report a case of precursor B-cell lymphoblastic lymphoma/leukemia showing the classic t(8;14) plus a complex variant translocation involving *BCL6*, *BCL2*, and *IGH*, t(3;14;18). The patient, a 36 y.o. female, presented with fatigue and a left neck mass. Excisional biopsy of the mass showed Burkitt-like morphology. A hypercellular bone marrow showed 94% blasts which expressed pan-B cell antigens, as well as CD10, *Bcl-2* and TdT, but not surface immunoglobulin. Interphase FISH using dual fusion probes specific for the t(8;14) and t(14;18) showed complex abnormal patterns in 82.5% and 92% of nuclei, respectively. Cytogenetic analysis of unstimulated bone marrow cultures confirmed the presence of both the 8;14 and the 14;18 translocations. Additional FISH studies showed that the 14;18 translocation was actually a three-way translocation that also involved the distal long arm of chromosome 3. An *IGH/BCL2* fusion signal was present on the derivative (der) chromosome 18, while the residual *BCL2* signal was observed on 3q, and the residual *IGH* signal on the der(14). Because these cells lacked a fusion signal on the der(14), which is usually the critical fusion event, and because FISH results implicated 3q27 as a possible partner in the 3-way translocation, a FISH assay using a break-apart probe specific for the *BCL6* locus was performed on the same metaphase cells. This assay confirmed involvement of *BCL6*, with translocation of 5 *BCL6* sequences to the der(14). Residual lymphoma was detected after 1 month of treatment and a second course of therapy was initiated, leading to complete cytogenetic remission after 6 weeks of therapy. However, one month later, her abnormal cytogenetic clone returned with additional evolution (a large marker chromosome). These results implicate *BCL6* as an important translocation partner with *IGH* in aggressive B-cell lymphoblastic lymphoma/leukemia.

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Loss of imprinting of LIT1 in colorectal cancers. *S. Nakano[1], M. Meguro[1], K. Murakami[1], H. Kugoh[1], H. Soejima[2], K. Higashimoto[2], T. Urano[3], T. Mukai[2], M. Oshimura[1].* 1) Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, Tottori University, Tottori, Japan; 2) Division of Molecular Biology and Genetics, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan; 3) Department of Biochemistry II, Graduate School of Medicine, Nagoya University, Nagoya, Japan.

Human chromosome region 11p15.5 contains several maternally and paternally imprinted genes, and the LIT1 locus acts as an imprinting center in the proximal domain of 11p15.5. Misregulation of LIT1 is associated with both Beckwith-Wiedemann syndrome and various cancers including colorectal cancer. To determine if LOI is a common in colorectal cancer, and to examine whether loss of imprinting (LOI) of the LIT1 is related to loss of methylation at a 11p15.5 region, we studied LOI of LIT1, IGF2 and H19, and DNA methylation of the KvDMR1, which is imprinting center at the 11p15.5, in 69 colorectal cancer tissues. LOI of LIT1, IGF2 and H19 were observed in 9/17 (53%), 11/20 (55% and 2/21 (9.5%) informative cases, respectively. LOI of LIT1 was only observed in tumor samples, while LOI of IGF2 was observed in both tumor and adjacent normal tissues in the majority of cases, suggesting that the both imprinting genes are not coordinately regulated. We carried out the LIT1 LOI analysis by RNA-FISH method, which does not require the sequence polymorphisms for the allelic expression profile. The analysis revealed that allelic expression patterns, i.e., LOI, imprinted and loss of expression of LIT1 were observed in 4, 1, and 1 cell lines, respectively. Methylation analysis performed at the KvDMR1. Two of the 4 cell lines with LIT1 LOI showed hypomethylation, while differential methylation was observed in the others. The cell line with the monoallelic imprinted pattern showed differential methylation, and the cell line with the loss of expression showed hypermethylation. Thus, there were two LIT1 LOI cell lines which maintained methylation status at the KvDMR1, suggesting that the KvDMR1 methylation status is not necessarily required for LIT1 LOI. We are currently studying other epigenetic profiles, such as histone modifications.

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Partial 3 or 5 IGH Deletions in Lymphoma. *J.H. Tepperberg, I. Gadi, V. Jaswaney, J. Kesler, L. Burgin, R. Royster, H. Waters, P. Papenhausen.* Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC.

Molecular cytogenetics, using an IGH break apart DNA probe, is an efficient, sensitive method for identifying most cases of B-cell Non-Hodgkins lymphoma. Lymphomas generally involve a reciprocal translocation of the heavy chain IGH gene, located at 14q32, resulting in the juxtaposition with an proto-oncogene, such as CMYC in Burkitt lymphoma, Cyclin D1 (BCL1) in Mantle Cell lymphoma or BCL2 in Follicular lymphomas. The result is transcriptional deregulation of the proto-oncogene and onset of disease. We report 4 cases (A-D) referred for FISH to rule out B-cell NHL in which either the 3 centromeric region or 5 telomeric portion of the IGH gene was deleted. The IGH break apart DNA probe (Vysis, Inc.) was designed with the 3 region flanking the constant region and the 5 variable segment distal to the J and D segment regions. In cases A-C, the 5 variable region was deleted suggesting that juxtaposition of the proto-oncogene with the 3 J region of IGH was sufficient to deregulate the proto-oncogene. Case A showed a reciprocal IGH-BCL2 gene rearrangement suggesting that the loss of the 5 gene segment was a secondary event. Case B showed a significant number of cells with a diminished 5 segment and an apparent deletion of the 5 region. The fourth case (D) showed a loss of the 3 region proximal to the IGH constant region. This suggests an oncogenic rearrangement with the 5 telomeric IGH region. Since the FISH probe does not cover the constant and VDJ regions, gene fusion could occur anywhere along this gene segment. A review of the literature revealed only one article describing a 5 IGH gene deletion in a patient with chronic lymphocytic leukemia associated with a poor prognosis. However, personal communications suggest that partial IGH deletions may be more common than previously reported, especially with the use of the break apart DNA probe. Histology, pathology, cytogenetic and FISH analyses of these lymphoma cases will be discussed with respect to prognosis.

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Quantum Dots Detection for HER2 Standard Reference Material. *Y. Xiao, P. Barker.* Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8311.

Approximately 30% of breast cancers have a cancer-specific genetic amplification of the HER2 gene. The result is that breast cancer cells in some patients have higher levels of HER2 receptor. Because of this, a newly developed drug (trastuzumab, or trade name Herceptin) can be used to treat HER2 positive patients. This drug targets tumor cells that have high HER2 receptor numbers. Two issues render HER2 diagnostic accuracy critical: 1) trastuzumab costs $40,000/year for treatment and 2) a similar receptor in normal heart tissue may also bind drug causing cardiotoxicity, and in some cases, death. Therefore, it is vital that the correct patients are identified for treatment. To avoid unnecessary risk and expense, it is also important to definitively ascertain those breast cancer patients for whom trastuzumab will not be effective. Unfortunately, the two diagnostic tests (FISH and IHC) for HER2 in breast cancer do not always identify the same patient subsets as candidates for drug therapy. So it is important to have HER2 standard reference material (SRM). We created the HER2 BAC clone as FISH probe(1), and designed anti-HER2 chicken antibody for IHC study. We used fluorescein and quantum dots labeled HER2 gene or receptor to directly and quantitatively measure HER2 gene or receptor in whole cells. Unlike organic fluorophores that photobleach during exposure, quantum dots are photostable, allowing quantitative bioimaging. By using 3D deconvolution imaging to capture all informative signals and minimize z-plane focal artifacts, we performed 3D quantitative analysis for HER2 gene copy numbers and HER2 receptor numbers in normal and breast cancer cells with tags detected with fluorescein- and quantum dot-streptavidin conjugates. We found that in breast cancer cells HER2 gene copy numbers was 18 times higher, and HER2 receptor numbers was 17 times higher than normal cells. (Supported in part by NIST HER2 SRM and the Office of Womens Health, NIH). References 1. Yan Xiao and Peter E. Barker, (2004) Nucleic Acids Research, 32 (3), e28.

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Skewed X chromosome inactivation is limited to BRCA1 mutation negative breast and ovarian cancer patients.

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A higher occurrence of skewed X chromosome inactivation (XCI) has been observed in breast and ovarian cancer with an early age of onset. As BRCA1 is implicated in both early onset breast/ovarian cancer and with the colocalization of XIST to the inactive X-chromosome, we hypothesized that skewed XCI would be limited to BRCA1 mutation carriers and associated with onset of disease. To test this, skewed XCI was evaluated using the methylation based PCR assay at the AR locus in peripheral blood samples from women with hereditary breast/ovarian cancer including: 42 affected BRCA1 mutation carriers, 16 unaffected BRCA1 mutation carriers, 30 BRCA1 mutation negative (22 BRCA2 carriers and 8 negative for BRCA1 and BRCA2 mutation testing) and 42 unaffected relatives. There was no increase in skewing above 90% in the BRCA1 mutation carriers (1 of 42 patients, 2.4%) when compared to unaffected BRCA-1 carriers (1/16; 6.2%) or control women (1 of 25 patients, 4.0%). However, there was an increase in extreme skewing in the BRCA mutation negative group (5 of 30, 17%) as compared to the BRCA1 carrier group (2/58,3.5%) (p=.04; Fishers test). Similar results were obtained using 75% as a cutoff for skewing. These results indicate a potential role for X-chromosome involvement in breast and ovarian cancer pathogenesis that does not involve BRCA1 mutations but may mirror BRCA1 cancer etiology.

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Molecular cytogenetic characterization of the 11q13 amplicon in head and neck squamous cell carcinoma. *C. Jin[1], Y. Jin[1], D. Gisselsson[1], S.W. Tsao[2], B. Strömbäck[1], M. Höglund[1], N. Mandahl[1], Y.L. Kwong[3], F. Mertens[1].* 1) Dept Clinical Genetics, University Hospital, Lund, Sweden; 2) Dept of Pathology, Faculty of Medicine, University of Hong Kong Hong Kong; 3) Dept of Medicine, Queen Mary Hospital, Pokfulam Road, Hong Kong.

Amplification of 11q13 DNA sequences or overexpression of *CCND1* is a common finding in head and neck squamous cell carcinoma (HNSCC), identified in about 30% of the cases. However, little is know about how the amplification is initiated or how the amplicon is organized. In order to study the structure of the amplicon in more detail and to learn more about the mechanisms involved in its initiation, prometaphase, metaphase, and anaphase fluorescence in situ hybridization (FISH) with 36 BAC clones spanning a 14 Mb region in chromosome bands 11q12.2 to 11q13.5 was undertaken in nine HNSCC cell lines with homogeneously staining region (hsr). The BAC clones covered oncogenes *CCND1*, *FGF4*, and *EMS1*. FISH analysis showed that the size of the amplicon varied among the 9 cell lines, the smallest being 2.32 Mb and the largest 8.34 Mb. The smallest overlapping region of amplification was approximately 1.64Mb, covering the region from BAC RP11-729E14 to BAC RP11-102B19. Eight cell lines could be further used to study the internal structure of the amplicon. A random pattern of amplified DNA sequences within the amplicon was found in all 8 cell lines. Even within the same cell line, different amplicon structures could be found in different cell populations, indicating that the mechanisms involved in the development of the amplicons in HNSCC were more complex than previously hypothesized. The frequent finding of inverted repeats within the amplicons suggests that breakage-fusion-bridge cycles are important in the initiation, however, the fact that such repeats constituted only smaller parts of the amplicons indicate that they are further rearranged during tumor progression.

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The simultaneous occurrence of Trisomy 1, 8 and 10 in Acute Myeloid Leukemia - a Case Report. *L. Karp, S. Gupta.* Laboratory Medicine, North Shore University Hospital, Manhasset, NY.

The presence of cytogenetic abnormalities with favourable and unfavourable prognostic significance is the strongest predictor of outcome in acute myelocytic leukemia and acute lymphocytic leukemia. Although well described in ALL, hyperdiploidy and teraploidy are rare cytogenetic abnormalities in MDS and AML. We present a 84 year old male with history of lethargy and fatigue:hemoglobin 9.5g/DL, platelets $56x10^9$/L, leucocytes $26x10^9$/L with 11% neutrophils, 75% lymphocytes, 4% monocytes and 3% eosinophils. Bone marrow examination showed hypercellular bone marrow with immature infilterate, erythroid hyperplasia, dyserythropoises, dysplastic megakaryocytes, focal lymphoid aggregates and increased iron stores. A minimal myeloperoxidase positivity was observed, chloroacetate esterase was positive in maturing myeloid component, naphthyl butyrate esterase negative. Flow cytometery results showed 26% myeloblasts, positive for CD34, CD117, HLA-Dr, CD33, CD13, CD15 and CD4. A diagnosis of acute myeloid leukemia was made. Chromosome studies of bone marrow cells revealed a kayotype with 48,XY,+1,del(5)(q22q33),+8,+10,-17[18]/ 46,XY[2]. Within various subtypes of AML, trisomies affecting chromosomes other than 8 are relatively uncommon. In AML trisomy 10 as sole karyotypic abnormality is a rare finding and karyotype with trisomy 1 as only chromosome abnormality is not known. Hyperdiploidy is present in 18% to 36% of all cases of ALL, and has favourable prognostic significance. Only a few cases of hyperdiploidy are known in AML, with most frequent gain of chromosomes 8, 21 and 19. This is the first report of the simultaneous occurrence of trisomy 1, 8 and 10 in acute myeloid leukemia.

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Detection of cryptic abnormalities in pediatric precursor B-cell acute lymphoblastic leukemia using an interphase FISH panel. *K.D. Tsuchiya, C. Gray, D. Williams, K.E. Opheim.* Dept of Laboratories, Children's Hosp & Regional Med Ctr, Seattle, WA.

Cytogenetic results in precursor B-cell acute lymphoblastic leukemia (pre-B ALL) are critical for determining prognosis and treatment stratification. Despite the importance of detecting cytogenetic aberrations in pre-B ALL, these cases remain challenging due to difficulties in obtaining sufficient numbers of high quality metaphase cells for analysis. We have instituted an interphase FISH panel for the detection of chromosome abnormalities that have known or suggested clinical significance in pre-B ALL. This panel includes probes for identifying MLL, BCR-ABL, and ETV6-RUNX1 (TEL-AML1) rearrangements, and deletions of 9p21. Over a one year period, we have applied this panel to 50 consecutive cases of pre-B ALL (45 initial diagnoses and 5 relapses). The frequency of abnormalities for initial diagnoses by G-banded analysis alone was 76% (34 out of 45 cases). This frequency increased to 91% (41 out of 45 cases) when FISH was combined with karyotyping. Cases that were apparently normal by G-banding, but abnormal by FISH included four cases with an ETV6-RUNX1 fusion, two cases with loss of one ETV6 signal, and one case with an extra copy of BCR due to trisomy 22. Another FISH finding of potential significance consisted of 24% of cases (11 out of 45 cases) showing deletion of 9p21 (four cases with homozygous deletions), five of which would not have been detected without the use of FISH. In two of these patients, cryptic 9p21 deletions occurred in the setting of high hyperdiploidy with trisomies for chromosomes 4, 10, and 17. The latter finding usually indicates a good prognosis, while cytogenetically visible 9p abnormalities have been reported to confer an increased risk of treatment failure (Heerema et al., Blood 94:1537,1999). The impact of cryptic 9p21 deletions identified by FISH in the setting of otherwise favorable cytogenetic findings remains to be determined. In conclusion, while there is no substitute for thorough G-banded analysis, interphase FISH can increase the detection of potentially clinically significant cryptic cytogenetic abnormalities in pre-B ALL.

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FISH analysis of B-cell non-Hodgkin lymphomas with t(8;14;18): Concurrent translocation of BCL2 and MYC to the same IGH locus. *J. Sanchez, D. Kramer, A. Adeyinka.* Medical Genetics, Henry Ford Health System, Detroit, MI.

A t(14;18)(q32;q21) involving the IGH locus on 14q32 and BCL2 locus on 18q21 is associated with follicular lymphoma, whereas a t(8;14)(q24;q32) involving the MYC locus on 8q24 is strongly associated with Burkitt lymphoma and to a lesser degree with large cell lymphoma or multiple myeloma. A few cases of highly malignant leukemia/lymphoma carrying both a t(8;14) and a t(14;18) have been reported. In these cases, each translocation usually involves a different chromosome 14 homolog. We present molecular cytogenetic findings in three cases of B-cell NHL with t(8;14;18)(q24;q32;q21). Two cases had near-diploid karyotypes and one had a near-tetraploid karyotype. Bone marrow sample cells were analyzed using commercially available dual-color dual-fusion probes for MYC/IGH and IGH/BCL2. Interphase FISH analysis showed the presence of IGH-MYC and IGH-BCL2 fusions in all three cases. The derivative chromosomes were better characterized by metaphase FISH analysis of cells from the two samples with diploid karyotypes. They include a der(8)(8pter8q24::14q32::18q2118qter) with MYC-IGH and IGH-BCL2 fusion signals, a der(14)(14pter14q32::8q248qter) with MYC-IGH fusion signal and a der(18)(18pter18q21::14q3214qter) with IGH-BCL2 fusion signal. There was a normal chromosome 14 in each of these two cases. These FISH findings suggest that the t(8;14;18) was derived from a translocation between chromosome 8 and a pre-existing der(14)t(14;18)(q32;q21). Reports exist of a few lymphoma cell lines with cytogenetically detected t(14;18) or t(8;14;18) and simultaneous activation of both BCL2 and MYC, the t(8;14) being cryptic in the cell line with t(14;18). Presumably, the molecular consequence of the t(8;14;18) in the present set of lymphomas is the concurrent activation of both BCL2 and MYC, similar to tumors with coexisting t(8;14) and t(14;18) that involve separate IGH loci, as a pathway of tumorigenesis and/or tumor progression.

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Identification of a complete genomic BRCA1 deletion in six belgian families. *K. Segers, P. Germeau, V. Bours.* Dept Genetics, Ctr Hosp Univ, Liege, Belgium.

Inherited mutations in the BRCA1 and BRCA2 genes are known to confer a predisposition to breast and ovarian cancer. The vast majority of mutations found in the BRCA1 gene are point mutations or small insertions and deletions scattered over the whole coding sequence and the splice junctions. Large deletions and duplications including one or several exons of the BRCA1 gene have also been described. Here we describe the complete loss of one copy of the BRCA1 gene at the genomic level. BRCA1 and BRCA2 are studied in our laboratory. A search for gross genomic rearrangement is first performed by MLPA. If normal, the screening for point mutation is done by DHPLC and sequencing. Nine patients representing six families and presenting a particular pattern for the BRCA1 gene were identified during the MLPA screening. All the amplification peaks were decreased suggesting a complete deletion of the gene. To date, no other patient presenting a complete BRCA1 deletion has been identified in Belgium. As our laboratory doesnt perform FISH analysis, several methods were used to confirm the absence of one BRCA1 copy. Seven common polymorphisms S1613G, S1436S, P871L, L771L, D693N, S694S and K1183R were studied. All the deleted patients were hemizygous for one allele. The highly polymorphic D17S855 microsatellite was also genotyped. All the deleted patients were hemizygous for one allele while the other members of the family that could be tested were all heterozygous. To exclude any error inherent to our laboratory, some samples were tested by MLPA in an independant laboratory. The results confirm those obtain in our lab. And finally, the samples were tested by a second MLPA kit that has been developed to confirm any abnormality observed with the first kit. The previous results were all confirmed by this second analysis. The absence of one BRCA1 copy is now confirmed. However, the extend of this deletion is not defined yet. We were not able to connect all our patients but the informations we have at the present time are poor. We can however speculate that all these families could be related to a common ancestor but we do not have any evidence for such a relationship.

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Interactions of sequence variants in IL-1 receptor-associated kinase 4 (IRAK4) and Toll-like receptor 1 (TLR1) genes increase prostate cancer risk. *J. Sun[1], K. K. Augustsson-Balter[2], F. Hsu[6], M. Hedelin[2], S.L. Zheng[1], H.O. Adami[2], B. Chang[1], J.E. Johnasson[4], T. Li[1], A.R. Turner[1], G. Li[1], W.B. Isaacs[5], J. Xu[1,6], H. Gronberg[3].* 1) Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Dept. of Medical Epi and Biosta, Karolinska Inst,Stockholm, Sweden;; 3) Dept.of Radiation Sciences, Oncology, Univ of Ume, Ume, Sweden;; 4) Dept. of Urology and Clinical Medicine, rebro Univ Hospital, Sweden and Regional Oncological Center, Univ Hopsital Uppsala, Sweden;; 5) Dept. of Urology, Johns Hopkins Medical Inst, Baltimore, MD; 6) Dept. of Public Health Sciences, Wake Forest Univ Sch of Med,.

Our previous research supports the hypothesis that the three inflammatory genes in the Toll like receptor (TLR1-6-10) gene cluster are involved in the prostate cancer risk. Interleukin-1 receptor-associated kinase 1 and 4 (IRAK1 and IRAK4) are critical components in the Toll/IL-1 receptor (TIR) signaling pathway, and they mediate downstream signaling following the binding of liands to TLRs. Considering the important role of IRAKs in the TIR signaling pathway, we proposed two hypotheses: First, sequence variants in IRAK1 and IRAK4 are associated with susceptibility to prostate cancer. Second, interactions between sequence variants in IRAK1/4 and TLR1-6-10 confer stronger risk to prostate cancer. To test these hypotheses, we performed a systematic genetic analysis of IRAK1 and IRAK4 genes by evaluating 11 single nucleotide polymorphisms (SNPs) (four in IRAK1 and seven in IRAK4) among 1,383 newly diagnosed prostate cancer patients and 780 age and residence matched controls in Sweden. While the SNPs in IRAK1 and IRAK4 alone are not significantly associated with prostate cancer risk, two SNPs in IRAK4, when combined with the risk genotype at TLR6-1-10, conferred a strong risk to prostate cancer. In particular, men with the combined genotypes of TLR1-6399 (CT/CC) and IRAK4-7987C/G (CG/CC) have a significantly increased risk to prostate cancer, with an OR of 9.68 (P = 0.03), when compared with men who have wild-type genotypes at these two SNPs. Our study supports a role of the TIR signaling pathway in prostate cancer risk.

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microRNA signature in breast cancer detected by a novel microsphere-based array, mirMASA. *Y. Feng[1], J. Han[2], Q. Yang[2], C. Lu[2].* 1) Dept Pharmacology, Emory Univ, Atlanta, GA; 2) Genaco Inc.

MicroRNAs (miRNAs) are an evolutionarily conserved novel class of small non-coding RNAs, which functions in gene silencing by posttranscriptional mechanisms. More than 300 miRNAs are encoded by the human genome. The expression level of many miRNAs is vigorously regulated during normal development. Moreover, emerging evidence indicates misregulation of miRNAs in human cancer, suggesting that miRNAs may play important roles in governing cell growth and development. We have developed a novel microsphere-based miRNA detection array, called mirMASA, which employs locked nucleic acids (LNA) to stabilize hybridization and allows direct capture of miRNAs in a multiplex reaction with digital output in real time. This technology offers greatly improved sensitivity and specificity as compared to conventional microarrays and northern hybridization. Further analysis reveals that mirMASA preferentially detects mature miRNAs over their precursors, and can distinguish closely related miRNA isotypes. Using mirMASA, we compared miRNA profiles in breast cancer specimens with that in normal breast tissue derived from the corresponding patients. Our results revealed a small group of miRNA that are abnormally expressed in all the cancer samples. More interestingly, these misregulated miRNAs gives two distinct ¡signature patterns¡ in different cancer samples, based on which a mathematical model is derived for diagnostic prediction of breast cancer. Hence, our studies established a highly sensitive and specific system for miRNA detection, which provided compelling evidence that misregulation of miRNA may serve as a signature for cancer genesis.

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An investigation of cholesterol biosynthesis by cortisol-producing, benign tumors of the adrenal gland. *C. Wassif[1], A. Horvath[2], A. Sterner[2], F. Porter[2], C. Stratakis[1, 2].* 1) NICHD/HDB/NIH, Bethesda, MD; 2) NICHD/DEB/NIH, Bethesda, MD.

Primary pigmented nodular adrenocortical disease (PPNAD) and other adrenal tumors (AT) may be caused by inactivating *PRKAR1A* mutations. cAMP/PKA pathway abnormalities have been found in sporadic cortisol-producing adenomas (CPAs) and the massive macronodular form of bilateral adrenocortical disease (MMAD). The cAMP pathway regulates many of the steps involved in cholesterol and steroid biosynthesis. Up-regulation of the enzymes involved in steroidogenesis has been shown in ATs; in this study, we investigated cholesterol biosynthesis by PPNAD (both assotiated with *PRKAR1A* mutations and caused by yet unknown genetic defects), CPAs, and MMAD. We analyzed 24 ATs for cholesterol and lathosterol content by gas chromatography/mass spectrometry, corrected for protein and tissue mass. We then looked at the expression of hydroxy-methyl-glutaryl-CoA reductase (*HMGCR*), LDL-receptor (*LDLR*), sterol delta-24 reductase (*DHCR24*), the reverse cholesterol transport-involved ATP-binding cassette, subfamily A, member 1 (*ABCA1*) and steroidogenic acute regulatory protein (*STAR*) in the tumor samples against mRNA from normal human adrenals. The lathosterol/cholesterol ratio of CPAs was 0.67 + 0.53 which was higher than that of PPNAD (0.12 + 0.06, $p<0.01$) and of MMAD (0.24 + 0.20, $p<0.05$). There were no differences between PPNAD and MMAD, and between ATs with and without *PRKAR1A* mutations ($P>0.5$). Consistent with the above, CPAs also had a tendency for higher expression of the *HMGCR*, and *LDLR* genes ($P=0.1$) and decreased expression of *ABCA1* ($P=0.06$); there were no differences between PPNAD and MMAD and tumors with and without *PRKAR1A* mutation. There were no differences in *DHCR24* and *STAR* expression between the various AT types. We conclude that sporadic cortisol producing tumors behave as cholesterol-starving tissues when compared to PPNAD, MMAD, and other ATs: increased cholesterol biosynthesis in the former is supported by a higher lathosterol/cholesterol ratio and *HMGCR* and *LDLR* expression, and a down-regulation of the cholesterol efflux-controlling *ABCA1* gene. *PRKAR1A* mutations do not appear to have distinct effect on these measures of cholesterol biosynthesis by ATs.

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Immunohistochemical Analysis of BRCA1-, BRCA2- and BRCAx-related Familial Breast Tumors. *R.S. Seitz[1], B.Z Ring[1], M.T. Schreeder[2], R. Beck[1], D.T. Ross[1], G. Chenevix-Trench[3], Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer.* 1) Applied Genomics, Inc., Huntsville, AL. and Sunnyvale, CA; 2) Comprehensive Cancer Institute Huntsville, AL; 3) Cancer & Cell Biology Division, Queensland Inst Med Research, Brisbane, QLD, Australia.

cDNA microarray studies have revealed reproducible breast tumor subtypes distinguished by characteristic gene expression signatures (Perou 2000; Sorlie 2001). Patients with BRCA1 mutations are highly enriched for expression of the 'basal-like' gene expression profile relative to sporadic cases (Sorlie 2003). Similarly, immunohistochemistry studies have demonstrated that BRCA1 associated tumors are associated with basal cytokeratin (CK5/6) expression (Foulkes 2003). We have generated over 700 novel antisera targeted by gene expression data in order to characterize the biologic and clinical diversity of breast cancer in paraffin blocks from tumor cohorts. We have identified a subset of forty-five reagents that distinguish reproducible heterogeneity in independent cohorts and have identified novel prognosticators for estrogen-receptor expressing breast carcinoma (Ross, SABCS, 2004). In order to explore the association between genetic predisposition to breast cancer and immunohistochemical subtypes of breast cancer, the Kathleen Cuningham Foundation Consortium has developed a pilot tissue microarray (TMA), containing 34 familial breast cancer cases (12 BRCA1-, 17 BRCA2- and 15 BRCAx-related) and 12 patients with sporadic tumors. A refined immunohistochemical profile of basal-like tumors will be compared to the protein expression profiles in BRCA1-associated patients. Immunohistochemical staining data with the panel of antisera will be presented to further explore whether a protein expression phenotype can distinguish familial from sporadic breast tumors, and whether there is an association between other mutation subtypes and immunohistochemistry phenotype.

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Estrogen-metabolizing enzyme polymorphisms and risks of breast cancer versus fibroadenoma. *E. Corder[1], L.A. Hefler[2].* 1) Demographic Studies, Duke Univ, Durham, NC; 2) Department of Obstetrics and Gynecology, Medical University Vienna, Vienna Austria.

Estrogen-metabolizing enzyme gene variants have been individually associated with risks of breast cancer (BRCA) and fibroadenoma (F) (Hefler et al., 2004). Distinct risk sets of polymorphisms, i.e. multilocus genotypes, have not been identified. To this end, we jointly investigated 10 candidate genotypes for BRCA (n=393) and F (n=154) patients and for control subjects (n=1936), cross-classified according to age (< age 50, 50-64 years, age 65+). The data analytic approach was a form of latent classification called grade-of-membership analysis (GoM) (Woodbury et al., 1978). The following 10 SNPs were analyzed using sequencing-on-chip technology via a solid-phase polymerase chain reaction assay performed on oligonucleotide microarrays: catechol-O-methyltransferase Val158Met G-->A, 17-beta-hydroxysteroid dehydrogenase type 1 vIV A-->C, cytochrome P-450 (CYP) family 17 A2 allele T-->C, CYP1A1-1 MspI restriction fragment length polymorphism (RFLP) T-->C, CYP1A1-2 Ile462Val A-->G, CYP19-1 Trp39Arg T-->C, CYP19-2 Arg264Cys C-->T, CYP19-3 Cys1558Thr C-->T, steroid-5-alpha reductase type 2 Val89Leu G-->C, and vitamin D receptor BsmI RFLP. Three GoM groups were requested. They were labeled as I, II & III and differed in disease frequencies (BRCA, F): I: 4%, 31%; II: 45%, 0%; III: 13%, 0%. They had distinct multilocus genotypes. Briefly, certain genotypes were limited to group II: CYP17(3), CYP1A1_1(2,3), CYP1A1_2(2,3), and CYP19_3(3), others to group I: COMT(3), SRD5A2(3), and VDR(3). Individual-level membership, i.e. resemblance, scores were used to predict disease status in logistic models [OR (95% CI)], according to age group. Group II membership predicted BRCA risk: 0.7 (0.3-1.6), 29 (15-59), 3.0 (1.0-9), respectively. Group I membership predicted F risk: 8.2 (4-17), 25 (10-68), n/a. We conclude that distinct sets of polymorphisms in genes that metabolize estrogen predict BRCA and F, most effectively (ORs > 25) for disease occuring in late middle age.

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Gene-expression profiles predict survival of patients with stage I non-small cell lung cancer: Results from a meta-analysis of four microarray studies. *Y. Lu[1], R. Yao[1], Z. Sun[2], W. Lemon[1], M. Watson[1], R. Govindan[1], P. Yang[2], Y. Wang[1], M. You[1].* 1) The Alvin J Siteman Cancer Center,Washington Univ. in St. Louis, St. Louis, MO; 2) Department of Health Science Research, Mayo Clinic, Rochester, MN.

Lung cancer is the most common cancer with the lowest overall 5-year relative survival rate of any tumor type. Patients diagnosed with stage I lung cancer have an extremely variable prognosis, ranging from relapse or death within 6 months to disease-free survival beyond five years. Microarray expression profiling has been widely used to identify genes related with lung cancer subtypes and development. To extract maximum value from the available microarray data, we applied a meta-analysis to search for commonly differentially expressed genes related to survival time (less than 3 years and greater than 5 years), cancer subtypes (ADC and SQCC), cancer stage (T1N0 and T2N0) and their interactions in lung cancer. We obtained data sets from four different microarray studies on lung cancer. We selected a consensus set of 4905 genes measured in all of the four studies and performed systematic bias adjustment in these data using Distance Weighted Discrimination (DWD). Meta-analysis identified 105, 181 and 28 genes related to survival, cancer subtype and stage respectively in the combined data, which were consistently altered in all of the four studies. Of the genes identified as differentially expressed, several were validated using RT-PCR. Functional annotation of these genes revealed that genes associated with cell growth and/or maintenance potentially determine survival time in lung cancer. Additionally, we used a penalized Cox regression model to predict survival after surgery for patients with stage I lung cancer and evaluated its performance in prediction by splitting the data into training and testing sets and using the time dependent receiver operating characteristic (ROC) curves. Kaplan-Meier curves showed very significant difference ($p<0.001$) in overall survival between high-risk and low-risk groups. Our findings provide a strategy to predict which stage I lung cancer patients may benefit from a surgical approach.

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Statistical and computational analysis of missense substitutions in BRCA2. *S.V. Tavtigian[1], A.M. Deffenbaugh[2], S. Monnier[1], L. Barjhoux[1], F.J. Couch[3].* 1) IARC, Lyon, France; 2) Myriad Genetic Laboratories, Inc., Salt Lake City, USA; 3) Mayo Clinic College of Medicine, Rochester, USA.

Genetic testing of high-risk cancer susceptibility genes contributes to the medical management of individuals who may have inherited risk of one or more cancers. Mutation screening of the breast and ovarian cancer susceptibility genes BRCA1 and BRCA2 is one such widely used genetic test. Clinical testing for deleterious mutations in these genes achieves very high sensitivity but also detects many unclassified variants, most of which are missense substitutions. We have developed in silico techniques that allow us to survey large numbers of missense substitutions in a specific gene, divide the substitutions into sets that are highly enriched for neutral variants or highly enriched for deleterious variants, and estimate average risks associated with the sequence variants in each defined set. Applied to BRCA1 or BRCA2, the basic requirements for the analysis are: a table of missense substitutions observed during mutation screening of high-risk individuals, including observational data such as the number of times that each substitution was observed, the number of times it was observed in an individual who was also found to carry a high risk mutation in that gene, and the number of times it was observed in an individual who was also found to carry a high-risk mutation in the other gene; and an informative protein multiple sequence alignment of the gene of interest. Although risks attributable to these two genes are similar, analysis of substitutions has focused on BRCA1. Here we present the first large-scale analysis of missense substitutions in BRCA2. Using a multiple sequence alignment of 9 full-length BRCA2 orthologs including sequences from pufferfish and sea urchin, we have analyzed all 1,143 missense substitutions that were observed during a series of 60,000 full sequence BRCA2 tests. As observed previously for BRCA1, we find that many of the missense substitutions falling at highly conserved positions in the BRCA2 alignment are likely to be deleterious and that the longer the period over which the position has been invariant, the stronger the evidence that this is so.

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Genome-wide expression analysis identifies cholesterol homeostasis genes as downstream targets of progesterone in ovarian surface epithelial cells. *J.E. Willett-Brozick, J.A. DeLoia, J. Patterson, L.C. Hsu, C.B. Wilcox, B.E. Baysal.*
Ob/Gyn, Univ Pittsburgh Sch Med and Magee-Womens Research Institute Pittsburgh, PA.

Background and Methods: Ovarian cancer, with a lifetime incidence of approximately 1%, accounts for more deaths than all other gynecologic malignancies combined. Ovarian cancer most often derives from ovarian surface epithelial cells. Several lines of evidence strongly suggest that progesterone exposure protects against ovarian cancer. However, the underlying mechanisms of this protection are incompletely understood. Here, we established short term *in vitro* cultures of non-neoplastic ovarian surface epithelial cells from six subjects, exposed the cells to progesterone (10^{-6} M) for five days and performed transcriptional profiling with oligonucleotide microarrays containing over 22,000 transcripts.

Results: We found that, in three of the six cultures, transcripts encoding 14 cholesterol biosynthesis enzymes, including the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, insulin-induced gene 1, low density lipoprotein receptor, ABCG1, endothelial lipase, stearoyl- CoA and fatty acid desaturases, long-chain fatty-acyl elongase, and MAC30 were upregulated; steroidogenic acute regulatory protein and ABCC6 were downregulated by progesterone. We confirmed the microarray results for a subset of the genes by quantitative RT-PCR analysis, and in one of three additional ovarian surface epithelial cell cultures. The cultures derived from a BRCA1 mutation carrier and another subject with early-onset breast cancer showed no evidence of transcriptional response to progesterone.

Conclusions: These findings indicate that progesterone regulates a very broad network of genes involved in cholesterol homeostasis in certain ovarian surface epithelial cells and provide new insights for understanding the protective role of progesterone against ovarian cancer.

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Clear cell ependymoma is characterized by trisomy 19. *E. Rousseau[1], M.M. Ruchoux[2], M. Vikkula[1], C. Godfraind[3], the two last authors contributed equally.* 1) Lab of Human Molecular Genetics, Christian de Duve Institute, Brussels, Belgium; 2) Lab of Pathology, CHU-Lille, France; 3) Lab of Pathology, University of Louvain and St Luc Hospital, Brussels, Belgium.

Ependymal tumors present a broad spectrum of clinical and histological subtypes. Few genetic clues have been reported suggesting that these different tumor subtypes follow independent genetic pathways during tumorigenesis. We describe a new genetic association to one ependymal tumor subtype reinforcing this concept as well as providing a new diagnostic tool. A series of 149 paraffin-embedded ependymal tumors were analysed by microsatellites in order to detect chromosome 9 monosomy, which has been implicated in 10-15% of ependymal tumors. Ten tumors presenting this abnormality were identified. They all presented histologically compact appearance. They bore a rich vascular network that consisted of regularly dispersed, geometrically branching capillaries. Tumor cells were regularly distributed and their cytoplasm could appear as clear (n=4). Areas of classical ependymal architecture were observed in some tumors. These tumors were classified as clear cell ependymomas. These tumors and additional ones, some of which lacked monosomy 9, but with similar histological findings, were further studied by array-CGH (Spectral Genomics). This allowed us to observe trisomy 19 in all tumors. Additional recurrent aberrations were found to involve chromosomes 1, 7, 11, 13, 17 and 20. Correlation between a genetic anomaly and ependymal tumors has never been described. Meanwhile, other cerebral tumors, including small cell glioblastomas and oligodendrogliomas, have been associated with, EGFR amplification and 1p/19q deletion, respectively. Such correlations are important tools for diagnosis. They also suggest the genetic pathways implicated in tumorigenesis. (catherine.godfraind@anpg.ucl.ac.be).

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Central nervous system atypical teratoid/rhabdoid tumor in a patient with Beckwith-Wiedemann syndrome. *N. Unanue[1], J. Biegel[1], F. Zhang[1], K.L. Ciprero[1], R. Weksberg[2], A. Judkins[3], B. Lange[4], J. Belasco[4], E.H. Zackai[1].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada; 3) Pathology Core Laboratory, Children's Hospital of Philadelphia, Philadelphia, PA; 4) Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA.

A patient was diagnosed at 10 month of age with Beckwith Wiedemann Syndrome (BWS) based on the findings of macrosomia (birth weight and birth length > 90th percentile), macroglossia, umbilical hernia, facial hemangioma and anterior ear creases. Molecular confirmation of BWS was obtained from methylation analysis of chromosome 11p15. These studies revealed hypomethylation of the maternal copy of LIT1, which silences the expression of a number of maternal genes, including CDKN1C. The child was followed by serial abdominal ultrasounds and AFP levels to rule out abdominal tumors, which were normal. At 20 months of age, after a head CT scan due to a sinus infection, he was noted to have a large fourth ventricle mass. The biopsy revealed an atypical teratoid/rhabdoid tumor. Molecular genetic analysis demonstrated a mutation in the INI1/hSNF5 rhabdoid tumor suppressor gene at chromosome 22q11.2. A C472T point mutation in exon 4 is predicted to result in an ArgStop codon change, and premature truncation of the protein. This was accompanied by loss of the wild type allele. This is the first case of an AT/RT seen in association with BWS, and suggests that patients with BWS may be susceptible to a variety of embryonal tumors not limited to rhabdomyosarcoma, Wilms tumor and hepatoblastoma. Previously reported loss of heterozygosity studies of renal rhabdoid tumor (Schofield et al) has implicated both loss of 22q11.2 and 11p15.

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The relationship between BRCA1 mutations and uterine serous papillary carcinoma in Ashkenazi Jewish women. *R.D. Legare, E.K. Brown, J.S. Wilbur.* Program in Women's Oncology, Women & Infants' Hospital, Brown University Medical School, Providence, RI.

Introduction: Several studies report a possible correlation between BRCA1 deleterious germline mutations and uterine serous papillary carcinoma (USPC) in Ashkenazi Jewish women. These studies outline a total of eight cases of women diagnosed with USPC who were also found to have inherited a BRCA1 mutation. Four women had the 187delAG mutation, and four women had the 5382insC mutation, which are two of the three most common mutations found in Ashkenazi Jewish individuals. Also, of the women who were diagnosed with USPC, twelve had a previous history of breast carcinoma, and sixteen had at least one first-degree relative with breast or ovarian cancer.

Case Study: This case report initially presented with a 51 year old Ashkenazi Jewish woman who was diagnosed with left breast DCIS at age 43 and ovarian cancer at age 50. She pursued comprehensive cancer genetic counseling and tested positive for the BRCA1 mutation 187delAG. Her paternal and maternal lineages were both suspicious for harboring this deleterious mutation. Since her mother had died of USPC, site specific BRCA1 gene analysis was able to be performed on her paraffin tumor block which was found positive for the 187delAG mutation confirming maternal inheritance.

Conclusion: This abstract reports another case of USPC in a heterozygote BRCA1 Ashkenazi Jewish woman. Therefore, USPC may be a manifestation of hereditary breast ovarian cancer syndrome (BOCS) in Eastern European Jewish patients and should be included in routine pedigree analysis. Further, a history of uterus carcinoma in this cohort should necessitate thorough review of pathology to rule out USPC. A larger study of USPC is necessary to substantiate its relationship to BOCS and may possibly impact the consideration of hysterectomy in individuals pursuing prophylactic oophorectomy.

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Review of Chronic Lymphocytic Leukemia cases with Ig/MYC translocation. A Burkitts lymphoma moniker translocation t(8;14)(q24;q32) in a Chronic Lymphocytic Leukemia patient had a cryptic deletion in the MYC region. *K. Reddy[1], R. Satyadev[1], D. Bouman[1], M. Hibbard[1], G. Lu[1], R. Paolo[2].* 1) US Labs, Irvine, CA; 2) Cancer Center of the Rockies, Fort Collins, CO.

A 53-year-old male was diagnosed with chronic lymphocytic leukemia [CLL]/small cell lymphoma [SLL] following splenectomy. The spleen and bone marrow [BM] showed 93% and ~70% of the viable cells were monoclonal lambda B-cells co-expressing CD5 with CD20, CD19, CD23, CD22, CD38, and low FMC-7. The smear showed a marked increase in small, mature lymphoid cells, with <2% prolymphocytes. The BM karyotype was 46,XY,t(8;14)(q24;q32),-18,+mar[3]/46,XY[27]. FISH: atypical interphase result with IgH(green)/MYC(red) dual fusion signal probe of one fusion, 2 green and one red signals in 70% of the cells and the expected t(8;14) pattern with MYC dual red-green split apart probe in 62% of the cells, indicated a cryptic deletion. Sequential FISH on a GTG-metaphase with t(8;14)showed a single fusion signal on derivative chromosome 8 and only a green signal on der(14) for IgH/MYC dual fusion probe and a green signal on der(14), red signal on der(8) and fusion signal on the normal chromosome 8 for MYC split apart probe. Hence, the apparently balanced t(8;14) had a cryptic deletion in between the red and the green regions flanking the MYC gene of the MYC split apart probe or 128,585,631 bp-130,226,339 bp [~1.6Mb] from 8pter. The single IgH/MYC fusion on der(8), green signal on der(14) and the consistent small MYC signal in the fusion compared to the normal MYC signal in interphases denoted a break and loss of the distal MYC probed region i.e. breakpoint to 129,141,156 bp and probably included the MYC gene. Post-chemotherapy, a decrease in the disease load, with the residual B-cells ~10% of the total population and aberrant FISH signal patterns for IgH/MYC and MYC split apart probes in 8% and 10% interphases, respectively was observed. The karyotype was 47,XY,t(8;14),-18,+2-3mar[cp2]/46,XY[18]. The rarity of t(8;14) in CLL, plus the cryptic deletion in the MYC gene region in our patient with CLL suggests an agressive clinicopathological role for MYC translocations in CLL.

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Automated Detection and Analysis of Circulating Tumor Cells. *M.W. Kilpatrick[1], F. Tafas[1], A. Seppo[1], I. Ichetovkin[1], Y. Kim[1], S. McGregor[2], G. Yardy[2], W.F. Bodmer[2], P. Tsipouras[2].* 1) Ikonisys Inc, New Haven, CT; 2) Cancer Research UK, WIMM, Oxford, UK.

Identification and analysis of rare circulating carcinoma cells has great potential; for detection of disease recurrence or minimal residual disease following treatment, or screening for malignancies. The challenge to the utilization of rare tumor cells diagnostically is to be able to accurately detect, quantify and analyse cells present in small numbers in a large, complex cellular background. The *Ikonisys*^{TM} platform is a customized system developed specifically for cell identification and analysis by automated fluorescence microscopy. All steps including slide loading, exposure setting, focusing, scanning, image capture and analysis, and slide unloading are accomplished in a completely unattended manner. This allows analysis of samples for the presence of rare cells avoiding complex purification procedures which a) risk loss of the cells being sought, b) are often unsatisfactory for scanning millions of cells or c) create unresolved clusters of normal and cancer cells. Initial experiments using dilutions of cultured tumor cells in a normal lymphoblastoid cell line, identified as few as 1 cell in 10^6 background cells. Samples were stained with two antibodies, Cam5.2 (anti-cytokeratin 8,18,19) and AUA1 (anti-EpCAM). Analysis of 20ml peripheral blood samples from 3 prostate cancer patients identified Cam5.2 +ve/AUA1 -ve cells in 2 patients, but identified 7 Cam5.2 +ve/AUA1 +ve cells in a patient with bone metastasis and extensive lymph node involvement. The automated slide scanning algorithms identify potential tumor cells at 5x magnification, based on the presence of Cam5.2 signal, and image all such cells at 50x for both Cam5.2 and AUA1 signal. In addition, slides can be scanned following combined antibody and FISH staining, and both antibody and FISH signals imaged at 50x, demonstrating the potential for further analysis of identified tumor cells. To date, the time-consuming search for rare cells makes it impractical to phenotype circulating tumor cells in depth. Further development of the *Ikonisys*^{TM} platform has great potential in the screening and diagnosis of cancer.

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Linkage analysis and comparative genomic hybridisation in a large familial prostate cancer pedigree. *L.M. FitzGerald[1], J.D. McKay[1,2], J. Stankovich[3], D. Challis[4], J. Brohede[5], J. Slavin[6], G. Hannan[5], R. Thomson[1], S. Quinn[1], D. Venter[1,7], J.L. Dickinson[1].* 1) Genetic Epidemiology, Menzies Research Institute, Hobart, Tasmania, Australia; 2) International Agency Against Cancer Research, Lyon, France; 3) Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Australia; 4) Histopathology Department, Royal Hobart Hospital, Hobart, Tasmania, Australia; 5) CSIRO Molecular Science, North Ryde, Sydney, New South Wales, Australia; 6) Department of Anatomical Pathology, St. Vincent's Hospital, Melbourne, Australia; 7) Mater Medical Research Institute, South Brisbane, Australia.

The complex genetic nature of familial prostate cancer (PC) has meant that the identification of the genes predisposing towards this disorder has proven challenging. The study of single large pedigrees provides considerable power to detect linkage, and also a degree of genetic homogeneity, both crucial to the elucidation of disease genes in complex disorders. We have combined the two technologies of SNP array analysis and Comparative Genomic Hybridisation in a large familial PC family originating from Tasmania, Australia. Analysis of the high-resolution GeneChip Human mapping 10K microarray, followed by targeted microsatellite genotyping, provided further evidence for linkage to the centromeric region of chromosome 5 (5p13-q12). Eight cases from the pedigree inherited a shared haplotype in this region and Simwalk2 analysis of microsatellite data gave a Statistic B score of 1.88 (p=0.013). High resolution BAC a-CGH in the same pedigree identified recurrent somatic genomic loss on 7p21 and 19p13.3, regions that have been identified in previous PC CGH studies. One patient who contributed to the evidence for linkage at 5p13-q12, also showed a gain of genetic material across this same region. These findings provide further evidence for a potential candidate prostate cancer gene on chromosome 5 and present insights into the somatic events of tumourgenesis.

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Incidence of genomic markers detected by FISH and their correlation with CD38 in B-cell chronic lymphocytic leukemia. *G. Lu, A. Anthony, C. Yue.* Department of Pathology, US LABS, Irvine, CA.

B-cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia. Studies on characteristic genomic markers of B-CLL have made significant progress in the diseases diagnostic and prognostic evaluation. The most frequent markers detected by FISH are deletions at 13q14, 17p13/P53, and 11q22-23/ATM, trisomy 12 as well as IgH rearrangements. High CD38 expression, originally described as a surrogate for unmutated IgVH genes, has also recently been used to evaluate aggressive B-CLL. Correlation of the chromosomal abnormalities with CD38 expression has been studied; however, there has been an evidence of discordant results between the few groups in the literature. The aim of this study was to establish the correlation of the different genomic markers with CD38 expression. This study included a total of 160 cases of B-cell CLL with FISH evaluation, conventional cytogenetics study, and flow cytometric analysis. Of the 160 cases, 107 (66.9%) were found to be FISH positive, whereas only 34 (21.3%) showed clonal abnormalities detected by conventional cytogenetics technique. Of the 107 FISH abnormal cases, as the sole or as one of the multiple changes, 11q/ATM deletion, 13q Deletion, 17p/P53 deletion, trisomy 12, or IgH rearrangement, was observed in 12, 41, 13, 21, and 17 cases, respectively. CD38 expression correlated with each genomic entry showed a low mean for 13q deletion or 17p deletion but particularly high for IgH rearrangement. Using > 30% as cut-off, we evaluated each FISH marker as the sole abnormality for positive CD38 correlation. This study showed (1) FISH studies in B-CLL were significantly sensitive over conventional cytogenetics (consistent with the literature); (2) 17p/P53 deletion is not significantly correlated with CD38 expression compared to the other markers; and (3) IgH rearrangement either as the sole abnormality or as part of the changes showed the most significant correlation with the cell membrane marker CD38 in B-CLL ($p<0.005$). We therefore suggest that IgH rearrangement could be a better marker for B-CLL prognostic evaluation, and should be included in the B-CLL FISH panel.

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Expression of PITX1 Homeobox Gene Transcripts in Oral Squamous Cell Carcinoma by In Situ Hybridization.

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Oral cancer accounts for at least 5% of all types of cancer worldwide and almost 95% of these cases are squamous cell carcinoma (OSCC). Due to the proximity of development and cancer, several studies are focusing on the participation of homeobox genes, a family of developmental regulators, in different types of cancers. Recently, PITX1 homeobox gene, related to development of stomodeum and of hind limbs, was found in OSCC cell lines through cDNA microarray analysis, but the expression of its transcripts in OSCC tissues has not been demonstrated yet. Therefore the purpose of the present study was to analyze the expression of PITX1 transcripts in OSCC and adjacent non-tumoral tissues using in situ hybridization. For such study, samples obtained from Hospital das Clínicas of São Paulo, Brazil, were divided in two parts for both RNA extraction with TRIzol and fixation in 4% paraformaldehyde. Transcripts were first amplified by RT-PCR and then localized by in situ hybridization (ISH) with specific digoxigenin-labelled riboprobes. Results showed that transcripts were amplified in 86,2% of the samples. ISH reactions showed that epithelial cells expressed PITX1 transcripts with a signal frequently intense in the non-tumoral adjacent epithelium. OSCC cells in general were weakly stained when comparing with non-tumoral epithelium. These results demonstrate the presence of PITX1 transcripts in OSCC and suggest that this gene may be involved in oral carcinogenesis.

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Polymorphisms in Phase I and Phase II Enzymes and their Implications on Leukemia Susceptibility in Caucasian, Hispanic, and African-American Children. *R. Swinney.* Dept of Pediatrics, UT Southwestern Medical Center, Dallas, TX.

Preliminary research has suggested the role of polymorphisms in phase I and Phase II enzymes (CYP450, NAT, GST) as etiological factors in the development of childhood leukemia. No study has investigated differences in mutation effects in the Hispanic, African-American and Caucasian populations. Therefore, we developed this case control study to determine if single nucleotide polymorphisms (SNPs) in these enzymes contribute to novel leukemia susceptibility in these ethnic communities. Methods: DNA samples were obtained from 285 children diagnosed with B-cell leukemia at a local pediatric hospital, and matched controls from non-leukemic children were obtained from blood draws at the same hospital. Controls were matched to cases by sex and ethnicity with variable match ratios. The controls and cases were examined via allele discrimination testing for SNPs in the Phase I (CYP450) and Phase II (GST, NAT) metabolizing enzyme genes. Results: The analysis demonstrated that the CYP1A1*2A allele had a borderline effect (^{2} = 3.5285, p = .0603) and CYP1A1*2B had a significant effect (^{2} = 4.3325, p = .0374) on cancer susceptibility. In Hispanic patients, this effect was accentuated with significant values for CYP1A1*2A (^{2} = 9.2447, p=.0098) and CYP1A1*2B (^{2} = 8.4260, p = .0148). In secondary analysis, the homozygous wild-type and heterozygous variants were combined, and compared to the homozygous variant cases in a dichotomous fashion. This revealed an increased effect on the entire cohort by CYP1A1*2A (p = .0112, OR = 2.34) and CYP1A1*2B (p = .0035, OR = 4.15). The effect on the Hispanic cohort was also accentuated for CYP1A1*2A (p = .0027, OR = 3.72) and CYP1A1*2B (p = .0043, OR = 4.3). Discussion: Our study suggests that SNPs in CYP1A1 have a significant impact on leukemia susceptibility in children, especially those children of Hispanic background. Although many of the other polymorphisms were not individually significant, there remains the possibility of numerous synergistic/antagonistic interactions between these polymorphisms. Therefore, further research is needed to explore the interactions between these genetic factors and leukemia susceptibility.

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Factors associated with an increased frequency of CDKN2A mutations in melanoma-prone families. *A.M. Goldstein, Melanoma Genetics Consortium (GenoMel).* Genetic Epidemiology Branch, DCEG,NCI,NIH,DHHS, Bethesda, MD.

CDKN2A is the major high-risk malignant melanoma (MM) susceptibility gene. Several variables have individually been reported to be associated with an increased frequency of CDKN2A mutations including many MM patients in a family [#MM/fam], multiple MM tumors in a patient [MPM], early median age at MM diagnosis in a family [MedAge], and pancreatic cancer [PC]. GenoMel, comprising most major familial melanoma research groups, conducted a study to explore the relationship between these four factors and mutations in CDKN2A. Families, tested for CDKN2A mutations, with at least three confirmed MM patients were eligible. The resulting sample included 384 families of which 151 (39%) had CDKN2A mutations. The frequency of mutations ranged from 20% (32/162) in Australia [Aus] to 45% (29/64) in North America [NA] to 57% (90/157) in Europe [Eur]. The four factors were evaluated across the 17 participating GenoMel groups (TOT) and in Aus, Eur, and NA. Mutation frequency increased significantly as #MM/fam increased in all groups. Mutation frequency was highest in Eur (41% for 3 MM/fam to 93% for 6 MM/fam) and lowest in Aus (12% for 3 MM/fam to 48% for 6 MM/fam). The frequency of mutations increased as MPM increased for all groups. For NA and Eur, 80% of families with 2 MPM patients had mutations. There was a significant relationship between PC and mutations in TOT, NA, and Eur but not in Aus (p=0.38). Only 3/9 Aus families with 1 PC had mutations versus 82% of families from NA (9/11) and Eur (32/39). All groups showed a significant increase in mutation frequency as MedAge decreased. Simultaneous evaluation of the four factors showed differences across the groups. In TOT, MedAge, MPM and PC, but not #MM/fam, were significantly associated with mutation. For Aus, MedAge, MPM and #MM/fam, but not PC, were associated with mutation. For NA, only MedAge and MPM were significantly associated with mutation; for Eur, only MPM and PC were related to risk of a mutation. Differences in #MM/fam, MedAge, MPM and PC may reflect distinct host, genetic, or environmental risk factors. GenoMel is exploring these factors to understand the variation across geographic regions.

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Gamma Delta T-Cell Lymphoma versus Large Granular Lymphocytic Leukemia- A Clinical Challenge. *J. Lester, S. Haq, A. Moussa.* Internal Medicine, University of Oklahoma, Tulsa.

BACKGROUND This case challenged our interpretation between Large Granular Lymphocytic Leukemia (LGL) and Gamma Delta T-cell Lymphoma. LGL is a rare leukemia with a median age onset at 55 years having characteristic morphology, autoimmune disorders and indolent clinical course in females. Gamma Delta Positive T-cell Lymphoma, is an rare aggressive non-Hodgkin's lymphoma variant found in young males. **CASE PRESENTATION** A 53-year-old female with a 3 year history of pancytopenia, recurrent upper respiratory infections and SLE presented with worsening cytopenia. The CT scan of chest/abdomen/pelvis revealed hepatosplenomegaly with lymphadenopathy. Blood smear showed pancytopenia with a minor population of large granular lymphocytes (RBC 3.2/l, Hb11gm/dl, Platelets 86X10 9/l, WBC 1.0, Neuts 0.1, Lymphs 80). Bone marrow biopsy revealed 40-50% cellularity with involvement by T-cell lymphoproliferative disorder of TCR-Gamma/Delta immunophenotype; positive for CD2,CD3, CD5 dim,CD7dim and negative for CD4, CD8, CD16, CD25, CD56 with variable CD57 expression. Molecular studies for T-cell receptor gene rearrangement showed no evidence of T-cell clonal population. The cytogenetics was normal diploid. The case was referred to M.D Anderson Cancer Centre for a second opinion. The final diagnosed with LGL. She was treated with cyclosporine for 8 months and responded well. **DISCUSSION** This highlights the clinical spectrum of LGL in the presence of an unusual immunophenotype. The patient was CD8- and CD3+. CD 8 negativity is not consistent with LGL; yet she had systemic signs and symptoms including fatigue, sweats, recurrent infections and arthritis, which are main clinical features of T-cell LGL. In addition the CT scan findings of hepatosplenomegaly and IgM/IgG monoclonal gammopathy supported the diagnosis of LGL, which represents 20% and 8% of the patients repectively. A female patient with an indolent clinical course and 72% lymphocytes in the peripheral blood also argues against the features of TCGD lymphoma . This case highlights the clinical spectrum of LGL and TCGD lymphoma and the importance of immunophenotypic and genotypic studies in diagnosis.

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Vascular Endothelial Growth Factor: A Serum Marker in Von Hippel Lindau. *B. Hirschman[1,2], J. Spaulding[2], L. Robinson[1], R. Brekken[2], G. Tomlinson[1,2,3], UTSW Clinical Working Group.* 1) Clinical Cancer Genetics, UTSouthwestern, Dallas, TX; 2) Hamon Center for Therapeutic Oncology, University of Texas Southwestern, Dallas, TX; 3) Childrens Medical Center, Dallas, TX.

Von Hippel Lindau (VHL) is a hereditary cancer predisposition syndrome caused by a mutation of the VHL gene at 3p25. The VHL protein participates in the proteolysis of hypoxia-inducible factor (HIF). Constitutive activity of HIF induces cellular responses only appropriate in hypoxia including the upregulation of angiogenic factors such as VEGF (vascular endothelial growth factor). VHL manifestations are typically hypervascular and include hemangioblastomas of the CNS, retinal angiomas, pheochromocytomas, clear cell renal carcinoma, and pancreatic and renal cysts. Although VHL is highly penetrant, manifestation type and timing are largely unpredictable. In this study, we are analyzing VEGF as a potential serum biomarker with aims to suggest a clinically applicable assay. Blood samples were collected from patients referred to Clinical Cancer Genetics at the University of Texas Southwestern Medical Center for VHL genetic testing. Statistical analysis of serum VEGF level has been performed on 78 of 98 patients accrued to date, ages 1 to 79 years from Caucasian and Hispanic families, based on availability of clinical information. Participants with familial risk who proved to be wildtype served as the control population. Serum VEGF level was measured by ELISA. A significant difference in serum VEGF level was found between VHL mutation carriers and wildtype controls ($p=0.0167$). Presence of manifestations at time of blood draw, with or without regard to location or quantity, failed to correlate to serum VEGF level. Age at blood draw was also not a significant factor in serum VEGF presence. Serum VEGF level above 247 pg/mL, the average value in an unselected adult population from Meo et al (2005), correlates to a significant odds ratio of carrying a VHL mutation ($OR = 5.14$, $p=0.0438$). In conclusion, while serum VEGF is increased in VHL patients, the increase is not correlated to manifestation site or quantity. Further studies are needed to determine the clinical value of VEGF in VHL.

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Variation in RUNX1 gene expression and its role in the development of leukemia in Down syndrome. *K.J. Rebro, M. Olivier.* Human & Molecular Genetics Center, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

Individuals with trisomy 21 have a 500-fold greater risk of developing acute megakaryblastic leukemia (AMKL), a normally rare subtype of acute myeloid leukemia (AML), than their chromosomally normal counterparts. Around 10% of newborns with trisomy 21 initially develop transient myeloproliferative disorder (TMPD), a transient form of leukemia characterized by a clonal population of megakaryoblasts. About 20% of the affected trisomy 21 children progress to AMKL within their first four years of life.

The molecular mechanisms underlying this trisomy 21-related leukemia are unclear; however, it is reasonable to postulate that the over-expression of gene(s) on chromosome 21 may play a critical role. RUNX1, a gene that is located on human chromosome 21, is a promising candidate gene since sequence variants within this gene are known to play a role in the development of other AML subtypes.

We examined sequence variants in the RUNX1 gene and the resulting linkage disequilibrium and haplotype structure of the gene region. In addition, we examined mRNA and protein expression in lymphoblast cell lines with different RUNX1 haplotypes. Our preliminary data show varying levels in RUNX1 expression in chromosomally normal individuals. We propose that these gene expression level differences of RUNX1 are enhanced in individuals with trisomy 21 due to the general over-expression of genes on chromosome 21, and that this alteration ultimately plays a crucial role in the development of TMPD and AMKL in a subset of children with Down syndrome.

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VHL germline mutations in Mexican patients with cerebellar hemangioblastoma. *A. Rasmussen[1,2], S. Nava-Salazar[3], P. Yescas[1], E. Alonso[1], R. Revuelta[4], I. Ortiz[4], S. Canizalez-Quinteros[5], MT. Tusié-Luna[5], M. López-López[6].*
1) Department of Neurogenetics and Molecular Biology; 2) Neurosurgery Division. Instituto Nacional de Neurología y Neurocirugía; 3) Doctorate in Biological Sciences Progra; 4) Biologic Systems Department, CBS Division, Universidad Autónoma Metropolitana; 5) Chemistry Faculty; 6) Molecular Biology and genomic medicine unit, Instituto Nacional de Ciencias Médicas y Nutrición; Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México. Mexico City, Mexico.

CNS hemangioblastomas (HB) are benign vascular tumors arising either sporadically or as a manifestation of von Hippel-Lindau disease (VHL), a hereditary cancer syndrome. We studied a series of patients with CNS HB and their families, in order to identify germline mutations in the VHL tumor suppressor gene, and to establish a predictive testing and scrutiny protocol. Methods: Patients admitted during 2002-2004 at the INNN for HB, were prospectively enrolled together with their at-risk family members. We performed the molecular analysis of the VHL gene by PCR and direct sequencing. All asymptomatic mutation-carriers underwent genetic counseling and tumor surveillance. Ninety-eight individuals were tested for VHL mutations: 23 symptomatic and 75 asymptomatic individuals, belonging to 16 families. Results: 7 of the families had definite clinical criteria of VHL disease, 5 had sporadic HB and 4 had CNS HB plus minor visceral signs. Molecular genetic testing allowed us to identify five germline mutations in 6 of the definite VHL families (sensitivity 85%) but none in the possible VHL and sporadic hemangioblastoma cases; four of these mutations had been previously described and one is a novel mutation present in two unrelated families. After mutation-carriers were identified, they underwent clinical screening and asymptomatic VHL-related lesions were identified in 43% of them. We conclude that genetic testing for mutations in the VHL gene is crucial in patients with CNS HB. The prompt identification of mutation carriers allows a multi-disciplinary screening protocol to decrease morbidity and mortality in these patients, while avoiding costly and invasive procedures for non-carriers.

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Long-term follow-up after genetic testing for HNPCC syndrome: impact on screening procedures and perception of genetic testing. *P.O. Chappuis[1,2], C. Gillabert[1], V. Membrez[3], A.E. Murphy[1], C. DeLozier[2], M. Gersbach[2], P. Hösli[1], P. Hutter[3], S.E. Antonarakis[2], A.P. Sappino[1].* 1) Division Oncology, Geneva Univ. Hosp., Geneva, Switzerland; 2) Division Medical Genetics, Geneva Univ. Hosp., Geneva, Switzerland; 3) Genetic Unit, Institut Central Hôp. Valaisans, Sion, Switzerland.

BACKGROUND: Regular colonoscopy in hereditary non-polyposis colorectal cancer (HNPCC) gene mutation carriers has been associated with an important reduction in the incidence of colorectal cancer. Understanding the factors influencing compliance with regular screening after genetic testing is a critical issue. Using a questionnaire, we investigated the long-term impact of testing on screening procedures and on satisfaction with the clinical process. METHODS: All HNPCC gene mutation carriers and non-carriers identified between 05.1995 and 03.2003 in 2 Swiss counselling centres were identified. An anonymous questionnaire was sent with 37 items exploring compliance with screening recommendations, basic understanding of HNPCC syndrome, and satisfaction with the counselling process. RESULTS: 198 individuals received informative HNPCC genetic testing results during the study period. 179 questionnaires were mailed; response rate was 85%. The average time of follow-up was 54 months. Answers from 61 carriers (26 m/35 f; mean age: 45.2 years; 55 *MLH1*/6 *MSH2* mutations) and 91 non-carriers (41 m/50 f; mean age: 44.7 years) were analysed. Only 5 (3%) persons did not correctly recall their genetic status. >80% of carriers and non-carriers followed all the screening recommendations. In the 12 months preceding the reception of the questionnaire, all mutation carriers had a colonoscopy. Carriers had significantly more colorectal screening than non-carriers ($P = 0.001$), due to the termination of surveillance in non-carriers. General understanding of the HNPCC syndrome was considered as good. Global satisfaction with the counselling process was >95%. CONCLUSION: In this retrospective long-term follow-up survey, compliance with screening recommendations and general satisfaction after genetic testing, both in carriers and non-carriers, were very satisfactory.

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Molecular analysis of mismatch repair defects in a diagnostic setting: immunohistochemical pattern predicts hereditary origin. *M. Ligtenberg[1,2], R. Willems[2], N. Arts[1], M. Schliekelmann[1], M. van Asseldonk[2], M. Goossens[2], K. Hebeda[2], H. van Krieken[2], N. Hoogerbrugge[1].* 1) Human Genetics, Radboud University Nijmegen, Nijmegen, Netherlands; 2) Patholgy,Radboud University Nijmegen, Nijmegen, Netherlands.

Germline mutations in mismatch repair (MMR) genes are a frequent cause of Hereditary Non-Polyposis Colorectal Cancer (HNPCC). More than 90% of HNPCC related tumors in patients with a germline mutation in one of the genes (*MLH1, MSH2, MSH6* or *PMS2*) show microsatellite instability (MSI). MSI also occurs in about 10% of the colorectal cancers of sporadic origin. Therefore MSI-analysis can be used as an overall marker of MMR deficiency, of either genetic or sporadic origin. The involvement of a specific MMR defect can be assessed by immunohistochemical(IHC) analysis and the analysis of *MLH1* promoter methylation. The latter is an indication of sporadic origin.

We have analyzed the IHC pattern and *MLH1* promoter status in 75 MSI positive tumors of patients that were counseled because of suspicion of HNPCC and matched the results with those of the mutation analysis. In 43 of the 75 patients (57%) a germline mutation in one of these genes was detected: 13 in *MLH1*, 22 in *MSH2* and 8 in *MSH6*. In 29 of 36 patients (81%) with tumors without staining of MSH2 and/or MSH6, a germline mutation was found. In 3 of 4 patients (75%) with tumors without PMS2, an *MLH1* mutation was detected. A germline mutation was detected in only 10 of 27 patients (37%) with tumors negative for MLH1 and PMS2. In 11 of 13 of these mutation negative tumors methylation of the *MLH1* promoter was detected.

These results show that a negative staining of the MMR proteins in absence of *MLH1* promoter methylation strongly predicts the presence of a germline mutation. Patients should be genetically counseled before IHC analysis of MMR proteins and determination of the methylation status of the *MLH1* promoter is performed, out of respect for the patients free choice to know or not to know the hereditary origin of their cancer.

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Impact of genetic counseling and DNA testing on individuals with colorectal cancer with a positive family history.
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What is the impact of a genetic evaluation for colorectal cancer susceptibility? We previously reported a study of individuals diagnosed with colorectal cancer at 60 years in a 5-county area of New York including Rochester. Subjects reporting at least one first- or second-degree relative with colorectal cancer were invited to receive genetic counseling and DNA testing. Of the 37 persons tested, six were found to have deleterious mutations in MSH2 or MLH1 and four had variants of uncertain clinical significance as previously reported (Loader et al., Genetic Testing 6:281, 2002). A deleterious mutation has since been found in a seventh subject in another laboratory.

To evaluate the impact of the testing experience, we obtained follow-up data on 36 of these 37 subjects. (1). *Colon cancer knowledge*. At 3 mos. after receiving their result, subjects knew more about colon cancer if they had more cancer of all types in their family (p=.02). At 12 mos., they knew more if they had received an abnormal result (whether a deleterious mutation or a variant of uncertain clinical significance) (p.001), were younger when DNA tested (p.01), or were younger when diagnosed with cancer (p.03). However, despite intensive counseling that the test was imperfectly sensitive, eight of those in whom no abnormality was detected thought an inherited susceptibility had been ruled out. (2) *Telling relatives*. All of those found to have an abnormality told relatives. Relatives of three subjects in whom a mutation had been found came to us for testing. (3) *Surveillance.* At 12 mos., surveillance for colon and endometrial cancer was more adherent if there were more total cancers in the family (p.05) or if the testee was more worried about cancer (p.05). (4) *Mental health*. Self-assessed mental health at 12 mos. was better for those who were married (p.05).

This study suggests that individuals undergoing a genetic evaluation for an inherited susceptibility to colorectal cancer remember their result, inform relatives of abnormalities found, and pursue recommended surveillance.

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Genetic and immunohistopathological evaluation of BRCA1 and BRCA2 unclassified variants. *G. Chenevix-Trench[1], S. Healey[1], S.R. Lakhani[1,2], R. Brinkworth[2], kConFab[3], A. Marsh[1], M. Brown[2], D.F. Easton[4], S. Tavtigian[5], D. Goldgar[6], A.B. Spurdle[1].* 1) Queensland Inst Medical Res, Brisbane, Australia; 2) University of Queensland, Brisbane; 3) Peter MacCallum Cancer Centre, Melbourne; 4) Strangeways Research Laboratory, University of Cambridge, UK; 5) International Agency for Research on Cancer, Lyon, France; 6) University of Utah.

About half the BRCA1 and BRCA2 variants detected in breast cancer families cannot be classified as pathogenic or neutral and are termed unclassified variants (UVs), representing a challenge for genetic counseling. Goldgar et al. (2004) developed an algorithm to classify UVs that incorporates data on co-occurrence with pathogenic mutations in the same gene, co-segregation with affected status, the Grantham Matrix Score and information on the evolutionary conservation to classify variants as neutral or pathogenic. We have identified 10 UVs in BRCA1 and 15 in BRCA2 from 37 pedigrees from the Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab). Segregation (using 421 germline DNAs), loss of heterozygosity (in 35 tumors), immunohistopathology and in silico conservation and Grantham Matrix analyses were conducted. The data were included in an updated version of the model which takes into account the characteristic immunohistopathology associated with pathogenic mutations in BRCA1, and the fact that ~85% of tumors from BRCA1 and BRCA2 carriers show loss of the wild type allele. Based on a threshold of 100:1 odds in favor of neutrality, we were able to classify 6 BRCA1 and 7 BRCA2 UVs as neutral. Four of these neutral variants were also identified in at least one of 180 controls analyzed, as was BRCA2 E2856A for which we found odds of 12:1 in favor of neutrality. Although two UVs (BRCA1 G1738R and BRCA2 D2723H, which was classified by Goldgar et al as pathogenic) reached odds in favor of causality of more than 250:1, none of them exceeded the 1000:1 threshold proposed for classification as pathogenic. Use of the updated model allowed the classification of four BRCA2 UVs which would have remained unclassified under the original version of the model.

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Identification of a Novel Frequent Large Genomic Rearrangement of *BRCA1* in High-risk Hispanic Families. *J. Weitzel[1], B. Hendrickson[2], V. Lagos[1], K. Lowstuter[1], M. Palomares[1], K. Blazer[1], K. Gonzalez[1], T. Scholl[2].* 1) City of Hope Cancer Center, Duarte, CA; 2) Myriad Genetic Laboratories, Salt Lake City, UT.

Background: Large rearrangements are estimated to account for 5-10% of all mutations in *BRCA1* and *BRCA2*. Prevalent founder rearrangement mutations have been described in European populations. We sought to identify rearrangements in the *BRCA* genes in a cohort of Hispanic patients. **Methods:** We identified 34 deleterious *BRCA* mutations via full sequence analysis among 110 unrelated high-risk Hispanic families enrolled in an IRB approved registry who underwent cancer risk assessment (CEBP 2005;14:1-6). To date, DNA from 45 of 76 patients without an identifiable mutation was subjected to multiplexed quantitative differential PCR (MQDP) for detection of large rearrangements. The assay consisted of multiplexed PCR reactions to assess gene dosage of all exons of *BRCA1* and *BRCA2* and the regions immediately 5 and 3 of the genes. Positive results were confirmed by long range PCR. **Results:** All 45 subjects had a history of breast cancer (1 with DCIS); 6 had bilateral breast cancer. Average age of first breast cancer diagnosis was 37.7 years. Reported ancestry included 29 Mexican, 6 Central American, 2 South American, 1 Spanish, and 7 of mixed country of origin. Mean pre-test *BRCA* mutation probability was 25.4%. In 3/45 (6.7%) families MQDP analysis identified an apparent deletion of *BRCA1* exons 9-12, indicated by a 50% loss of signal. Long range PCR resulted in the generation of a 2.5kb product in these samples, consistent with a deletion event of 15.1kb. All three families reported Mexican ancestry and were unrelated through at least a 4-generation pedigree. **Conclusion:** We identified a novel large deletion in three unrelated families of Mexican ancestry, suggesting founder effect. The frequency was 2.7% of the 110 high-risk families screened for *BRCA* mutations, bringing the overall prevalence of deleterious mutations to 33.6%(37/110). This *BRCA1* rearrangement may account for a substantial proportion of high-risk Hispanic families, and should be included in all subsequent studies of this ethnic group.

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The 471delAAAG mutation and C353T polymorphism in the RNASEL gene in sporadic and inherited cancer in Israel. *R. Gershoni-Baruch[1], E. Dagan[2], Y. Laitman[3], E. Friedman[4].* 1) Inst Human Genetics, Rambam Medical Ctr and Bruce Rappoport School of Medicine, Technion, Haifa, Israel; 2) Inst Human Genetics, Rambam Medical Ctr and Dept of Nursing, University of Haifa, Israel; 3) Susanne-Levy Gertner Oncogenetics Unit and Dept of Gynecology, Sheba Medical Ctr,Tel-Hashomer, Israel; 4) Susanne-Levy Gertner Oncogenetics Unit, Sheba Medical Ctr,Tel-Hashomer and Sackler School of Medicine, Tel-Aviv University, Israel.

To evaluate the role of RNASEL 471delAAAG germ-line mutation in cancer predisposition a total of 1011 individuals including 294 Jewish men with prostate cancer (190 Ashkenazim), 361 Jewish Ashkenazi women with breast and/or ovarian cancer and 356 unaffected Ashkenazi women were investigated. Sequence anomalies in a single RNASEL gene amplicon using either of DGGE or DHPLC, restriction methods and sequencing were sought. The 471delAAAG mutation was detected in a single male with prostate cancer (1/294 and 1/190 of the Ashkenazi patients - 0.3% and 0.53%, respectively), in two ovarian cancer patients (2/141 -1.4%) one with and one without BRCA1 mutation, and in a single woman of 242 healthy controls (0.41%). An abnormal DHPLC profile identical to the one produced by the 471delAAAG mutation was noted in 23 additional women: 2 (0.94%), 16 (7.8%), and 5 (2.6%) from among carriers, non-carriers, and controls, respectively. Sequence analysis disclosed a silent polymorphism in Valine at codon 118: c.353 C->T. The rate of this polymorphism was significantly elevated in high-risk non-carrier women (16/205; 7.8%) either with breast and ovarian cancer (13/148; 8.8%) or asymptomatic (3/57; 5.3%) than in BRCA1/2 mutation carriers (2/209; 1.0%) ($X=11.670$; $p<0.001$). The 471delAAAG mutation occurs only rarely in unselected Israeli prostate cancer patients and in high-risk Ashkenazi breast and/or ovarian cancer patients, at rates that do not differ from that of the general population. A silent polymorphism in the RNASEL gene that mimics the abnormal DHPLC pattern seems to occur more prevalently in high-risk Ashkenazi breast and/or ovarian cancer patients, non-BRCA1/2 carriers, perhaps being in linkage disequilibrium with a pathogentic mutation.

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Genomic and epigenetic profiling of p53 and non-p53 Li-Fraumeni syndrome tumors reveals multiple hits in the p53 pathway. *R. Krahe[1], M. Tuna[1], L.L. Bachinski[1], B. Zhang[1], S. Colella[1], R. Luthra[3], G. Lozano[1], L.C. Strong[2].* 1) Sect. Cancer Genetics; 2) Clinical Cancer Genetics; 3) Dept. Hematopathology, Univ. Texas M.D. Anderson Cancer Ctr., Houston, TX.

Common dogma in cancer genetics holds that multiple hits in the same pathway are redundant, thus unlikely. Using genomic and epigenetic profiling, we show that p53 Li-Fraumeni syndrome (LFS) tumors can harbor multiple hits in other p53 pathway members. Moreover, p53 and non-p53 LFS tumors share hits in p53 pathway members. LFS is clinically and genetically heterogeneous. Most cases (>70%) are due to mutations in p53 (17p13.1). CHK2 (22q12.1) is a minor predisposing locus, and we recently mapped a third locus to 1q23. In both p53 and non-p53 LFS, there is evidence for risk heterogeneity within and between kindreds, suggesting additional risk modifiers and factors, including epigenetic alterations. We profiled genomic DNA from primary tumors and matching constitutive DNA of 10 LFS patients (6 with p53 mutations and 4 from non-p53 kindreds), using BAC array CGH (Spectral Genomics) and the 100K SNPChip (Affymetrix) to identify patterns of global DNA changes or allelic imbalance, and PyroMethA to ascertain the methylation status of 11 known tumor suppressor genes. We resequenced known mutations in BRAF, KRAS and HRAS. Our global approach revealed several interesting patterns: for example, 5 of 6 p53 LFS patients showed loss of RB1 (13q14.2) and CCNA1 (13q13.3), while 2 of 4 non-p53 LFS patients showed gain for the same region. Five of 6 p53 LFS patients also showed loss of the p53-activated target 14-3-3e (17p13.3). Tumors of 3 unrelated p53 LFS patients showed concomitant loss of 4p13-p11, 9pter-p21.1, 10q11.1-q24.2 and 13q13-q14.2, and gain of 1q21.1-q24.1, suggesting the shared involvement of genes in these regions in p53-associated LFS. Six genes (TP73, RASSF1, BRCA1, HIC1, ESR1 and HIN1) showed significant tumor-associated promoter hypermethylation. RASSF1 hypermethylation was exclusively associated with soft-tissue sarcomas. In conclusion, different combinations of genetic and epigenetic alterations appear to act together to dysregulate a limited number of cellular pathways in LFS tumorigenesis.

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Analysis of AKT-TOR-HIF pathway component genes in patient tumors: associating mutations with targeted therapy. *M.L. Nickerson[1], J.S.. Troy[1], R. Thiagarajan[1], K.B. Walters[1], T.K. Price[1], M.D. Geimer[1], J.A. Durocher[1], S. Lilleberg[1,2].* 1) Discovery Services, Transgenomic, Gaithersburg, MD; 2) Discovery Services, Transgenomic, Omaha, NE.

Though the molecular details are only partly understood, it appears possible that a common signaling pathway including AKT, TOR, and HIF may integrate environmental cues in order to allow a coordinated, holistic response by a cell. The presence of multiple tumor suppressors that down-regulate signaling by AKT-Tor-Hif pathway members, including PTEN, TSC 1 and 2, and VHL implicate disregulation of pathway signaling in multiple types of cancers and hamartoma syndromes. Although individual genes have been analyzed in specific cancers and syndromes, the mutation status of the pathway as a whole in cancer remains to be determined. This study examines the spectrum of mutations and polymorphisms in genes encoding pathway components in DNA from early stage prostate and breast tumors. A comprehensive analysis of each tumor was achieved by examination of 240 amplicons from PIK3CA, PTEN, AKT 1-3, TSC 1 and 2, Tor, HIF 1-3, VHL, and IRS 1 and 2. A novel mutation detection strategy was utilized which consisted of three independent methods to identify polymorphisms in PCR amplicons from tumor DNA: Surveyor Nuclease enzymatic cleavage of mismatches, DHPLC separation of heteroduplexes, and double-stranded fluorescent sequencing. Approximately 230 variants will be presented, some of which have therapeutic implications. Functional analyses will confirm the causative nature of these genetic variants and their contributions to tumorigenesis. Other groups have focused on gene families for analysis of somatic mutations in tumors, such as kinases and phosphatases. This study takes a signaling pathway approach to group gene targets for analysis. Analysis of pathway genes may prove useful since mutations in genes encoding upstream pathway components may be treatable using therapeutic agents directed against downstream targets. Patient health can hopefully be improved by correlating specific mutations with treatment options that delay cancer onset and progression while maximizing response and outcome.

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Pilot to assess parental receptiveness to the creation of a youth cohort (LEGACY) within the Ontario familial breast cancer registry (OFBCR). *G. Glendon[1], D. Hanna[1], C. Mulhall[1], P. Ritvo[1], A. Thompson[1], I.L. Andrulis[1,2].* 1) Ont. Cancer Genetics Network, Cancer Care Ontario, Toronto, ON, Canada; 2) Mt. Sinai Hospital- Samuel Lunenfeld Research Institute, Toronto, ON, Canada.

Breast cancer risk is influenced by genetic and environmental factors that exert effects prior to adulthood. The OFBCR is a population-based breast cancer registry consisting of 2500 families with varying degrees of family history. Expanding this cohort to include family members under the age of 18 would allow the investigation of important early events in breast cancer. Specifically, hormonal, biological and genetic factors that are associated with the intermediate endpoints of puberty, menarche, body growth and composition, and breast tissue characteristics. We have conducted semi-structured, in-depth interviews with 20 adult-parent participants of the OFBCR to gain a detailed understanding of their interests and concerns regarding their offspring's enrollment in a proposed OFBCR youth cohort study entitled LEGACY (Lessons in Epidemiology and Genetics of Adult Cancers from Youth). Interviewees were females diagnosed with breast cancer between January 1996 and 1999 who have daughters between the ages of 5 and 17. Interview domains include the extent of familial cancer disclosure, receptiveness and attitudes to LEGACY enrollment, preferred level of interaction between daughters and LEGACY staff, minimum age for enrollment and all other issues identified in the interview. Qualitative analysis utilizing grounded theory was conducted from the transcribed interviews. All 20 (100%) interviewees identified LEGACY as a good idea and stated that they would be willing to enroll their daughters if only data were collected. Seventeen (85%) stated that they would enroll their daughters if data and blood were collected. All 20 (100%) stated they would be willing to act as intermediary for enrollment purposes, questionnaire data collection and blood collection. Average minimum age for minor enrollment was 9.6 and 10 years respectively for a data only vs. data and blood study with a range of age 5 to age 16. We will conduct 60 additional female interviews as well as 40 with randomly selected male spouses.

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Examining Ontario Familial Breast Cancer Registry (OFBCR) proband participants response to providing kin contact information for study enrollment purposes. *A. Thompson[1,2], G. Glendon[1,2], D. Hanna[1,2], L. Anson Cartwright[2], K. Turnbull[2], K. Keast[2], M. Edwards[2], N.F. Boyd[2], I.L. Andrulis[1,2,3].* 1) Cancer Care Ontario - Ontario Cancer Genetics Network 620 University Ave. Toronto, ON, Canada M5G 2L7; 2) University Health Network - Ontario Familial Breast Cancer Registry 610 University Avenue, Toronto, Ontario M5G 2M9; 3) Mt. Sinai Hospital- Samuel Lunenfeld Research Institute 600 University Avenue, Toronto, ON, Canada M5G 1X5.

The OFBCR is the Ontario site of the NCI funded Breast Cancer Family Registry consortium. It is a familial cancer registry that collects cancer family history, epidemiological breast cancer risk information, and biological samples from individuals with incident breast cancer and selected kin. The purpose of the present study was to determine the reasons why some OFBCR proband participants were unwilling to provide kin contact information for study enrollment purposes. We identified these proband participants and performed telephone interviews to assess reasons for non-disclosure of kin contact information and to determine if these kin were now able to participate in the OFBCR. If the proband participant agreed to provide kin contact information, addresses were obtained over the phone or returned via a mailed list after the interview. Four hundred eighty five participants, with a total of 2308 eligible kin, were contacted by phone. One hundred forty seven participants agreed to kin involvement for selected kin. Two hundred thirty six kin addresses were obtained over the phone and 27 kin addresses have been returned by mail for a total of 263 addresses collected thus far. Reasons for refusal to provide kin contact information included 28% refused with no reason, 21% lost contact with kin, 17% kin not interested, 10% deceased/unwell/too old, 8% location/language barrier, 7% not willing to ask kin again, 3% don't get along with kin, 2% too busy, 4% other. We have demonstrated that some OFBCR proband participants that initially were unwilling to provide kin contact information for study enrollment purposes may reconsider this position over time.

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Exacerbation of transcriptional inactivity leads to epigenetic silencing of a tumor suppressor gene. *M. Ren, S. Pozzi, G. Bistulfi, S. Rossetti, G. Somenzi, N. Sacchi.* Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY.

Resistance to the growth inhibitory action of retinoic acid (RA), the bioactive derivative of Vitamin A is common in human tumors. One form of RA resistance has been associated with aberrant DNA methylation and silencing of the retinoic acid receptor beta 2 (RARB2) gene, whose transcription is regulated by RA. Understanding why RARB2 is prone to aberrant epigenetic silencing in human cells is critical for both prevention and early treatment of RA-resistance in human carcinoma cells. Here we show that an exacerbation of RARB2 transcriptional inactivity leads to heritable RARB2 DNA methylation and silencing. Further, we show that RARB2 epigenetic silencing leads to the development of RA-resistance and distinct features of tumor progression. This study serves as the proof of principle that RARB2 epigenetic silencing is consequent to an exacerbated transcriptional inactivity. Supported by the DOD-IDEA Award DAMD17-02-1-0432 (NS).

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Influence of glutathione-S-transferase (GSTM1, GSTP1, GSTT1) and cytochrome p450 (CYP1A1, CYP2D6) polymorphisms on numbers of basal cell carcinomas (BCCs) in families with the nevoid basal cell carcinoma syndrome (NBCCS). *R. Yang, R.M. Pfeiffer, A.M. Goldstein.* Dept DCEG, NCI, Bethesda, MD.

The nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant multisystem disorder with variable expression. NBCCS patients have varied susceptibility to basal cell carcinoma (BCC) development. Previous studies have demonstrated that polymorphisms of some metabolic genes encoding the cytochrome p450 (CYP) and glutathione-S-transferase (GST) enzymes influenced the numbers of BCCs in sporadic BCC cases. In this study, we genotyped and analyzed 152 members (69 affected and 83 unaffected) of 13 families with NBCCS for 7 polymorphisms in 5 metabolic genes including CYP1A1, CYP2D6, GSTM1, GSTP1, and GSTT1. GSTP1 Val^{105} and GSTP1 Val^{114} alleles were significantly associated with fewer BCC numbers (odds ratio $[OR]_{105}$ = 0.55, 95% confidence interval [CI], 0.35 to 0.88; OR_{114} = 0.20, 95% CI, 0.05 to 0.88). The Val^{105} allele demonstrated a dosage-dependent effect ($OR_{Ile/Val}$= 0.58, 95% CI, 0.34 to 0.88; $OR_{Val/Val}$= 0.34, 95% CI, 0.14 to 0.78). In addition, we observed fewer jaw cysts in carriers of the three p450 polymorphisms (CYP1A1m1, CYP1A1m2, and CYP2D6*4) ($OR_{CYP1A1m1}$ = 0.27, 95% CI, 0.12 to 0.58; $OR_{CYP1A1m2}$ = 0.25, 95% CI, 0.08 to 0.78; $OR_{CYP2D6*4}$ = 0.33, 95% CI, 0.18 to 0.60). Our data show that genetic variants might contribute to the variation in numbers of BCCs and jaw cysts observed in NBCCS families.

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Identification of genetic modifiers in a large BRCA1 family. *R.E. Ellsworth[1], R. Leach[2], L.R. Hoffman[1], C. Snyder[3], H.T. Lynch[3].* 1) Windber Research Inst, Windber, PA; 2) Teneo Sciences, Bethesda, MD; 3) Creighton University School of Medicine, Omaha, NE.

Although mutations in BRCA1 and BRCA2 represent the primary lesions in hereditary breast and ovarian cancer (HBOC) families, these mutations are marked by incomplete penetrance, as 15-20% of carriers never develop either cancer. In addition, the expressivity of these gene mutations varies with manifestation in breast, ovary, or both breast and ovary. To identify additional genes associated with dictating tumor location, we have collected DNA samples from 52 members of a large six-generation HBOC family with an IVS16-581del1014 BRCA1 mutation. Over 10,000 genotypes were determined for each individual using the GeneChip Mapping 10K Array (Affymetrix, Santa Clara, CA). Disease modeling using artificial intelligence-based algorithms was performed using Exemplar 2.0.0 (Teneo Sciences, Bethesda, MD). Allele frequencies of several SNPs were significantly different between carriers with cancer and those without. SNPs on chromosomes 13q33 and 18p11 could be used to correctly classify carriers with breast cancer, a SNP on chromosome 2q31 effectively separated carriers with ovarian cancer, and a SNP on chromosome Xp11 reliably identified carriers with breast and/or ovarian cancer($P < 0.05$). Genes from these regions include the RAB6C gene on chromosome 2q31, the ligase 4 (LIG4) gene on chromosome 13q33.3, variations in which have been associated with a protective advantage against breast cancer, the EPB41L3 gene on chromosome 18p11, and the ubiquitously expressed transcript (UXT), which is overexpressed in a number of tumor cell lines. Models can thus be designed in which alone, mutations in BRCA1 are not sufficient to promote breast cancer, but interactions between these candidate genetic modifiers and BRCA1 provides an environment hospitable to tumor development. Confirmation of these results using data generated from other BRCA1 families and identification of causative mutations will provide more effective diagnostic tools for counseling mutation carriers and determining optimal treatment regimens.

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Mosaicism confounds prenatal diagnosis of retinoblastoma: Exclusion of disease after linkage analysis predicted an affected fetus. *R.D. Clark[1], D. Rushlow[2], B.L. Gallie[2], S.O. Cox[3], A.L. Murphree[3].* 1) Division of Medical Genetics, Department of Pediatrics, Loma Linda School of Medicine, Loma Linda, CA; 2) Retinoblastoma Solutions, University Health Network, University of Toronto, Toronto, Canada; 3) Division of Ophthalmology, Retinoblastoma Program, Childrens Hospital Los Angeles, Los Angeles, CA.

We report a 2 generation family with retinoblastoma (Rb) in which undetected germline mosaicism led to misinterpretation of linkage analysis data for prenatal diagnosis.

A 37-year old pregnant G2 P1 female with a history of unilateral Rb at 21 months, presented for genetic counseling regarding the prenatal diagnosis of Rb in her fetus. She was the first affected member of her family although her only child had bilateral Rb. In 1998, RB1 gene sequence analysis, performed elsewhere, on the patients blood was normal and linkage analysis was done using polymorphic intragenic RB1 probes. In the current pregnancy, amniocytes expressed the at-risk RB1 haplotype and the patient was given a greater than 99% chance that her fetus was affected. RB1 mutation studies were then performed on her affected child, the patient, and her fetus.

The patients affected child had an RB1 mutation, R455X, in her blood. Using R455X-specific PCR, the patient was shown to be mosaic for this mutation. The amniocytes of the fetus did not show the R455X mutation. The newborn eye exam was normal.

Linkage analysis was misleading because, in the mosaic proband, some cells with the at-risk haplotype did not harbor the RB1 mutation. Mosaicism should be suspected when the first case in the family has unilateral Rb. For this reason, linkage analysis can be unreliable for the prenatal diagnosis of retinoblastoma in some families.

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Duplications of the MSH2 and MLH1 genes in Hereditary Non Polyposis Colorectal Cancer (HNPCC) / Lynch syndrome. *S. Baert-Desurmont[1,2], S. Frerot[1], C. Martin[1,2], E. Bessenay[1], F. Charbonnier[2], S. Olschwang[3], Q. Wang[4], M-P. Buisine[5], T. Frebourg[1,2].* 1) Department of Genetics, Rouen University Hospital, Rouen, France; 2) Inserm U614, Faculty of Medicine, Rouen, France; 3) Department of Genetics, Institut Paoli Calmettes, Marseille, France; 4) Molecular Oncology, Centre Léon Bérard, Lyon, France; 5) Laboratory of Biochemistry and Molecular Biology, Lille University Hospital, France.

Hereditary Non Polyposis Colorectal Cancer (HNPCC) or Lynch syndrome results from germline mutations of the DNA mismatch-repair genes MSH2, MLH1, MSH6 and PMS2. The systematic screening for genomic rearrangements in 332 HNPCC families without MSH2 or MLH1 point mutations, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments), allowed us to report previously that genomic deletions of MSH2 and MLH1 are respectively involved in approximately 10 % and 4 % of the HNPCC families fulfilling Amsterdam criteria. We identified 22 and 15 distinct exonic deletions of MSH2 and MLH1, respectively. We have now detected, using QMPSF, 8 distinct (including 5 novel) genomic duplications of MSH2 and MLH1: 6 duplications of MSH2, affecting respectively exons 1-2-3, exons 5-6, exon 7, exons 7-8, exons 9-10, and exon 11, and 2 duplications of MLH1, involving exons 2-3 and exons 6-7-8. These duplications were confirmed by MLPA (Multiplex Ligation-dependent Probe Amplification). The recent reports highlighting segmental duplications and copy number variation in the human genome led us to check that these duplications affecting MSH2 or MLH1 did not correspond to polymorphisms. Although genomic duplications of MSH2 and MLH1 are less frequent that genomic deletions, their presence should be considered in HNPCC families without detectable point mutations and their detection can easily be performed using QMPSF.

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Single cell gel electrophoresis (comet assay) to assess genomic instability in different esophageal pathologies. *Y. Ahuja[1], M. Vasavi[2], V. Bhavani[3], P. Shivani[2], K. Kishore[3], V. Kiran[2], Q. Hasan[2,3].* 1) VMRC, Hyderabad 500 004,India; 2) Dept of Genetics & Molecular Medicine, Kamineni Hospitals, L B nagar, Hyderabad 500 068, India; 3) Dept of Genetics, BMMRC, AC Guards, Hyderabad 500 004, India.

Cancer is a multistep process resulting from a series of genomic alterations that lead to the progressive malfunctioning of crucial mechanisms of cell growth, differentiation and death. Most human cancers are associated with genomic instability this was assessed in different esophageal pathologies using Single cell gel electrophoresis (SCGE) / Comet assay. 1ml of heparinised peripheral blood was collected from 124 individuals referred for endoscopy with upper GI tract symptoms. Clinical, personal and family history was collected. 63 samples with >85% cell viability by Trypan blue test were processed for SCGE analysis. Individuals were categorized into four groups based on clinical evaluation, endoscopic and histopathological reports into: 23 cases of cancer, 7 of premalignant lesions, 8 with inflammatory pathologies and 25 endoscopically normal individuals taken as controls. 40ul of blood was processed for comet assay by standard lysis, alkaline treatment and electrophoresis. 100 cells per patient were scored using the oculometer after silver staining. The mean percent of damaged cells was 37% in cancers, 30.7% in pre-cancers, 15.6% in cases with inflammatory esophagitis and 10.6% in controls. The number of damaged cells from controls to cancers was significant ($p<0.0001$). One-way ANOVA showed a significant increase in the comet tail length between the four groups ($p<0.005$) indicating a progressive increase in DNA damage from controls and esophagitis to precancers and cancers. DNA damage did not appear to have any association with smoking, tobacco chewing and alcohol consumption. Comet assay in peripheral blood reflects the DNA damage associated with pre-cancers and cancers of esophagus. Our study also indicates that the DNA damage seen was mostly due to the exposure to endogenous disease promoting agents in the tissue microenvironment rather than exogenous agents.

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Genealogy and genetic evidence linking AAPC kindreds to an American founder. *D. Neklason[1,2], J. Stevens[3], N. Matsunami[3], B. Otterund[3], J. Barlow[1], R. Burt[1,4], M. Leppert[3].* 1) Huntsman Cancer Institute; 2) Oncological Sciences; 3) Human Genetics; 4) Medicine; University of Utah, Salt Lake City, UT.

We previously reported four independently ascertained attenuated familial adenomatous polyposis (AFAP) kindreds with an identical mutation in the APC gene (exon 4: 426_427delAT) (Spirio et al, 1993). We hypothesize that these four kindreds are descendents of a common ancestor from America. We continue to expand and phenotype two of these kindreds, 353 and 439 (Burt et al, 2004) and here provide genealogy and genetic data to support their common heritage. The 353 kindred is from Utah with more than 4000 descendents spanning 8 generations. 490 members have been studied and 145 are mutation positive. The founding parents were born in Massachusetts and New York in the 1790s and were part of the Mormon emigration to Utah in the 1850s. The 439 kindred is from upstate New York spanning 6 generations. 99 members have been studied and 36 are mutation positive. The founding parents were born in New York in the 1830s. Using genealogic resources, ancestors from both kindreds have been traced to the same Massachusetts town in the 1600s, however the common ancestor has not been identified. Using the Affymetrix GeneChip 10K SNP array, we have found that the two kindreds share an identical haplotype of 31 SNPs spanning 6.48Mb across the APC region. This genetic data provides convincing evidence that these two kindreds descend from a common ancestor. To our knowledge, this mutation has not been reported outside of the USA, suggestive of an American founder. This founder may also be the ancestor of the other two kindreds originally described, but this has yet to be determined.

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Functional analysis of inherited genetic variants associated with hereditary colorectal cancer predisposition. *S. Perera, B. Bapat.* Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common form of inherited colorectal cancer. Germline mutations in the mismatch repair (MMR) genes lead to this autosomal dominant disorder, characterized by the development of cancers of the colon, endometrium, stomach, urinary tract, ovaries, small bowel and brain. 90% of known cases of HNPCC arise due to defects in the MMR genes *hMLH1* and *hMSH2*. Around 20-30% of MMR gene alterations reported to date are missense changes. In the absence of functional data, the pathogenic effects of these changes cannot be accurately interpreted. This poses problems for predictive genetics testing programs offered to HNPCC patients. Thus the aim of this project is to investigate the consequence of these alterations on the function of the protein and the efficiency of the MMR system. We have selected 5 unclassified variants in *hMLH1*: I19F, A128S, R265C, K618A and L749Q, based on their presence in functional domains, predicted effect and the evolutionary status of the respective amino acid residues. These alterations were created in plasmids containing wild type MLH1 cDNA by site-directed mutagenesis, and transiently transfected into the colon cancer cell line HCT116, which is deficient in hMLH1. Preliminary results indicate that both R265C and L749Q decrease the expression of hMLH1 compared to the wild type vector, however with R265C a more striking effect is seen. This novel observation will be verified by independent experiments. Quantitative RT-PCR assays are being used to determine the level at which the alterations affect the expression of the protein. Co-immunoprecipitation experiments are being carried out to address the possibility that these alterations disrupt interactions of hMLH1 with its partner proteins, and the effects of the alterations on the stability and turnover of the protein will be investigated using pulse chase assays. These studies will provide a better understanding of the function of MMR proteins and will help to decrease the ambiguity that arises in predictive genetic testing and counseling of HNPCC patients and their families.

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Prostate Cancer Specific Clinical Data on African-American and Caucasian Males in Louisiana: Impact of Family History. *D.M. Mandal[1], S.L. Halton[1], J.J. Hunter[1], O. Sartor[2], J.E. Bailey-Wilson[3], W. Rayford[4].* 1) Dept Genetics, LSU Health Sciences Ctr, New Orleans, LA; 2) Stanley Scott Cancer Center, LSU Health Sciences Ctr, New Orleans, LA; 3) NHGRI/NIH, Bethesda, MD; 4) Dept of Urology, LSU Health Sciences Center, New Orleans, LA.

Prostate cancer is a heterogeneous disease, the exact causes of which are still unknown. There are several significant risk factors identified, including age, race and family history. African-American men have an incidence rate of prostate cancer almost two times greater than Caucasian American men and in addition, they are two to three times more likely to die from this disease. Studies examining the disproportionately higher incidence and death rate of prostate cancer among African-Americans indicate that this dire impact might be attributed to an earlier onset of the disease and delayed diagnosis. The purpose of this study is to compare clinical/pathological characteristics of men with familial and sporadic prostate cancer in African-American and Caucasian males. Clinical records were reviewed for 100 prostate cancer (approx. 40 African-American and 60 Caucasian) cases identified with positive family history from 30 families and the clinical characteristics, including age at onset, Prostate Specific Antigen (PSA) values at the time of diagnosis, Gleason scores and clinical stages were compared with an equal number of prostate cancer cases with no family history. All sporadic prostate cancer cases were verified for family history of the disease. Statistical analyses and a detailed account of our data resources will be presented comparing clinical and pathological characteristics of men in both races, in conjunction with the use of family history information. Preliminary analyses show that there is a significant difference in the age at onset between familial and sporadic prostate cancer cases both in African-American ($p=0.002$) and Caucasian ($p=0.01$) patients. The result of the study will allow us to understand the characteristics of the disease in different ethnic groups recruited from Louisiana.

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Reclassification of 54 BRCA1 DNA sequence variants of uncertain significance as benign polymorphisms has positively affected test results for 1500 patients. *A.M. Deffenbaugh, D. Pruss, T. Judkins, T. Scholl, L.A. Burbidge, C.A. Frye, M. Frost, R. Iyer, J. Tazelaar, B.E. Ward, W.W. Noll.* Myriad Genetic Laboratories, Salt Lake City, UT.

Introduction: *BRCA1/BRCA2* genetic testing for hereditary breast/ovarian cancer is important in managing patients who may be at increased risk of cancer. Clearly positive or negative test results are usually obtained, but sometimes sequence variants of uncertain significance (UVs) are found, producing a clinically ambiguous test result. Efforts to gather additional information so that these variants may be reclassified as deleterious or benign are essential. All evidence currently available in the literature and in the Myriad database indicates that a person cannot carry biallelic deleterious mutations in *BRCA1*. Mouse studies indicate that absence of *Brca1* expression is embryonically lethal, and in more than 70,000 individuals tested, Myriad has never identified two deleterious mutations in *BRCA1* that are on opposite chromosomes. **Methods:** All *BRCA1* UVs identified as homozygous or *in trans* with a deleterious mutation through haplotype analysis or family testing were reclassified as genetic variants, favor polymorphism. If additional data were available, such as lack of co-segregation of the variant with cancer in families, variants were reclassified as polymorphisms. **Results:** 54 *BRCA1* variants previously classified as UVs were reclassified as favor polymorphism (31/54) or polymorphism (23/54). This has resulted in the reclassification of test results for 1505 patients and has reduced the percentage of patients tested for *BRCA1* and *BRCA2* who receive an overall test result of genetic variant of uncertain significance from 11% to 9.5%. The number of patients who carry only a *BRCA1* UV has decreased 43%. **Conclusion:** Reclassification of *BRCA1* UVs using the sole criterion that a UV identified *in trans* with a deleterious mutation is unlikely to be clinically significant has resulted in the clarification of genetic test results for more than 1500 patients. As data continue to accumulate regarding the clinical significance of UVs in *BRCA1* and *BRCA2*, additional reclassifications will be made.

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The Norwegian founder mutation, BRCA1:1135insA, occurs on different haplotypes in ethnically diverse populations. *N. Hamel[1], T.M. Rudkin[2], M. Galvez[2], F. Hogervorst[3], J.J.P. Gille[4], P. Møller[5], J. Apold[6], W.D. Foulkes[1,2,7].* 1) Dept Medicine and Research Institute, McGill Univ. Health Centre, Montreal, QC, Canada; 2) Dept Human Genetics, McGill Univ., Montreal, QC, Canada; 3) Netherlands Cancer Institute, Amsterdam, Netherlands; 4) Dept Clinical Genetics, VU Univ. Medical Centre, Amsterdam, Netherlands; 5) Dept Cancer Genetics, Norwegian Radium Hospital, N-0310 Oslo, Norway; 6) Center of Medical Genetics and Molecular Medicine, Haukeland Univ. Hospital, Bergen, Norway; 7) Cancer Prevention Centre, Sir M.B. Davis-Jewish General Hospital, McGill Univ., Montreal, QC, Canada.

Analysis of the chromosomal background upon which a mutation occurs can be used to reconstruct the origins of specific disease-causing mutations. The commonly observed BRCA1 mutation, 1135insA, has been previously identified as a Norwegian founder mutation, but it has also been observed in individuals with other ethnic backgrounds. In the BIC database, the 1135insA mutation is the 12th most common frameshift mutation occurring in BRCA1. To determine whether all carriers of the mutation share a common ancestry, we performed haplotype analysis of carriers from breast and ovarian cancer families with several different ethnic origins. We used four polymorphic microsatellite repeat markers located within and adjacent to BRCA1 for haplotype analysis. We found that the mutation occurs on several, clearly distinct haplotypes. Divergent haplotypes can sometimes be explained by recombination events following the appearance of the mutation. However, such events are unlikely to account for our observations given the proximity of the intragenic markers tested. While recombination events could have occurred between two of the markers, our haplotype results are not consistent with this scenario. The DNA sequence surrounding the mutation includes a run of seven consecutive adenines that may be prone to slippage during DNA replication. This may create a hot-spot within BRCA1 where mutations are more likely to occur than elsewhere in the gene. Our results suggest BRCA1:1135insA is a recurrent mutation that should be included in all targeted mutation panels, irrespective of the ethnic origin of the individuals tested.

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Characterization of a large germline deletion of the VHL gene: Alu-mediated mechanism underlying VHL rearrangements. *A. Casarin, M. Martella, R. Polli, E. Leonardi, L. Anesi, A. Murgia.* Pediatrics, Univ Padua, Padua, Italy.

Mutations of the VHL gene (3p25) are responsible of von Hippel-Lindau disease, an autosomal dominant familial cancer syndrome predisposing to the development of benign and malignant tumors, among which CNS and retinal hemangioblastomas, clear cell renal carcinomas and pheochromocytomas. At least 30% of the disease-causing mutations of the VHL gene are represented by large alterations. The identification of these mutations is not possible with commonly used PCR-based mutational scanning methods. Quantitative Southern blot analysis has been traditionally employed for the detection of complete or partial deletions and seemingly more complex rearrangements of this gene. With the combined use of quantitative Southern blot and Real-Time PCR analysis, developed as an alternative quantitative method for the detection of hemizygosity, we have studied 30 large alterations of the VHL gene. Aim of the study was to determine the exact nature of the mutations and possibly to characterize the boundaries of the deleted regions. The Real-Time PCR analysis demonstrated that all the VHL rearrangements detected by Southern blot were actually represented by large deletions. One of the samples was found to carry a deletion of about 2.2 Kb which included exon 2 and part of the flanking introns. A long-range PCR encompassing the deleted fragment showed that both the 5 and 3 break-points were located within Alu-repeats, present in a large number within the VHL coding sequence. This is the first characterization of the break-point junction of a large VHL gene deletion. Our data suggest that an Alu-mediated mechanism is likely to be responsible of the common occurrence of large alterations in the VHL gene.

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Genomic rearrangements of BRCA1 and BRCA2 genes in Belgian families with breast and/or ovarian cancers. *P. Hilbert, F. Cavallin, M. D'Amico, Y. Gillerot.* Human Genetics Center, Inst de Pathologie et Genetique, Loverval, Belgium.

Germline mutations in BRCA1 and BRCA2 genes cause predisposition to breast and ovarian cancers. Standard mutation detection screening based on PCR are aimed at identifying coding or splice site mutations but miss large rearrangements of the genes. Such rearrangements account for 5 to 30 % of mutations of BRCA1 gene, depending on populations studied. The availability of MLPA technology (Multiple Ligation dependent Probe Amplification) now allows the easy, fast and unexpensive screening of families for single or multiple exon deletion or amplification. To determine the frequency of such rearrangements in our at risk population, we tested all our mutation-negative patients by MLPA for BRCA1 and BRCA2 genes. On a total of 158 families, 34 carried evident mutations in either BRCA1 (21) or BRCA2 (13) and 9 (respectively 6 and 3) carried a variant of undetermined significance. On the remaining 124 patients without significant mutation, we found 7 genomic rearrangements, exclusively in BRCA1. Six that had been previously described were confirmed by PCR: one duplication of exon 13, one deletion of exon 22 and 4 deletions of exons 8 to 13 . The last one, previously undescribed, is a deletion of exon 23 that we further characterized (c.IVS22+664_IVS24-162 del 2282). No deletion nor amplification in BRCA2 was identified. Large genomic rearrangement of BRCA1 hence represent 17 % of the mutations in our breast/ovarian cancer families and 23 % if we consider only BRCA1 mutations. Interestingly, our results, obtained on patients from the southern part of Belgium, differ significantly from those obtained by another Belgian group in the Northern part of the country (2 %). We confirm that the search for genomic rearrangement is necessary in the BRCA1 and 2 screening process.

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Screening for APC mutations in the Iranian FAP patients. *M. Shahmoradgoli[1], O. Mueller[2], N. Kutzner[2], P. Rostami[1], H. Najmabadi[3], B. Noorinayer[1], M.R. Zali[1].* 1) Research Center for Gastroenterology and Liver Diseases, Taleghani Hospital, Shaheed Beheshti University of Medical Sciences, Tehran, Iran; 2) Max-Planck Institute for Molecular Physiology, Dortmund, Germany; 3) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

Germ-line mutations in the adenomatous polyposis coli (APC) gene are causative for familial adenomatous polyposis (FAP), a dominant inherited syndrome characterized by presence of multiple adenomatous polyps in the colon and rectum. In this study, we screened the coding region of APC gene for germ-line mutations in ten unrelated Iranian FAP patients. These patients showed classical FAP symptoms. Our study began with a non-radioactive protein truncation test (PTT) to screen all patients for protein truncating mutations in mutation cluster region (MCR) of the APC gene. Then, we analyzed all 15 exons of the APC gene in patients with negative PTT results by single-strand conformation polymorphism (SSCP) and by DNA sequencing. APC mutations were detected in five (50%) patients including three protein truncating and two missense mutations. Nonsense mutations found were C3925T (Q1303X), G3943T (E1309X) and C3962G (S1315X). The G3967C (E1317Q) and G2407A (G797S) were missense mutations detected in two other patients. Genotype-phenotype correlation has been also shown in our study. Furthermore, two novel silent polymorphisms, G4497A and A5898G, were found in our patients. Although we used a combination of PTT, SSCP and DNA sequencing techniques to screen coding region of the APC gene, no causative mutation has been found in five patients. Besides the possibility of missing some mutations due to imperfect sensitivity of SSCP in these patients, existence of mutations located outside the coding region of APC or involvement of other genes, should not be excluded. Based on our findings, APC mutations are responsible for at least 50% of FAP patients in Iran.

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***FH* mutation analysis, enzyme activity, protein modeling and early functional study in hereditary leiomyomatosis and renal cell cancer.** *M. Pithukpakorn[1,2], M.H. Wei[2], O. Toure[2], G.M. Glenn[2], L. Middelton[2], M. Merino[2], B. Zbar[2], L. Neckers[2], W.M. Linehan[2], J.R. Toro[2].* 1) NHGRI, and; 2) NCI, NIH, Bethesda, MD.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is the autosomal dominant predisposition to the development of leiomyoma of the skin and uterus and/or renal cancer. We previously showed that mutations in fumarate hydratase gene (*FH*) are associated with HLRCC. With 56 families to date, we have identified 31 different germline *FH* mutations, of which 9 were novel. These 31 mutations consist of 20 missense, 9 frameshifts (3 insertions and 6 deletions), 2 nonsense and 1 splice site. To date our *FH* mutation detection rate is 93% (52/56) in families suspected of HLRCC. Sixteen families shared the R190H mutation and four families shared the R58X mutation. Renal tumors were found in 32% (18/56) of families. The *FH* mutations associated with these 18 families with renal tumors consisted of R58X (3 families), R190H (4 families), L89S, S102X, R190C, H 275Y, S376P, Q396P, c.111insA, c.780delGC, c.1162delA, c.1339delG, c.138+1G>C. *FH* mutations were associated with a spectrum of renal tumors. LOH at the *FH* gene locus was found in 83% of HLRCC renal tumors. This finding supported the role of *FH* as a tumor suppressor gene. We also report for the first time the FH activity in cytosolic and mitochondrial fractions in cell lines from HLRCC patients. Lymphoblastoid cells (n=13) and fibroblasts (n=4) from the individuals with HLRCC had statistically significantly lower FH activity than normal control cells ($p<0.05$). There was no difference in FH activity between normal controls and 15 cell lines from patients with VHL and BHD. FH activity in fibroblasts from HLRCC patients was as low as in patients with FH deficiency but their urine organic acids were normal. In addition, we present the three-dimensional atomistic model of the human FH. Using siRNA to inhibit FH activity in culture cells, we found 2/3 reduction of FH enzyme activity in fibroblasts. We recently found that cellular accumulation of fumarate impairs hypoxia inducible factor (HIF) prolyl hydroxylase and leads to stabilization of HIF, suggesting that the VHL-HIF pathway may play role in tumorigenesis in HLRCC.

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Genetic Factors in African American Women with Breast Cancer. *T. Pal[1, 3], S. Vadaparmpil[1, 3], S. Narod[2], J. Betts[3], C. Miree[3].* 1) Department of Interdisciplinary Oncology, University of South Florida, Tampa, FL; 2) Center for Research in Women's Health, Toronto (Canada); 3) H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

BACKGROUND. Because a larger proportion of African-American women have early onset breast cancer, we hypothesized that a subset of these women may have a genetic cause of their increased risk, such as BRCA1 and BRCA2 (BRCA1/2).

METHODS. We are conducting a study investigating BRCA1/2 among African American breast cancer patients with a personal or family history suggestive of hereditary predisposition to breast cancer. To date, twenty-three women have been enrolled. The majority (n=9; 39%) were recruited from the Moffitt Breast Program physicians. Other sources of recruitment included: outside physicians (n=3; 13%), newspaper advertisements and brochures (n=3; 13%), FORCE organization/website (n=2; 9%), community or support groups referrals (n=4; 17%), and word of mouth (n=2; 9%). RESULTS. The average age of study participants and age at cancer diagnosis is 41 (SD=7.35) and 39 (SD=6.39), respectively. BRCA1/2 test results are currently available on 18 patients, and 1 (5.5%) was found to be a mutation carrier. Review of family history data showed that 9 (39%) had a first or second-degree relative with breast cancer, and 3 (13%) had a relative with ovarian cancer.

CONCLUSIONS. Our preliminary results suggest a small proportion of breast cancers in young African American women are due to the BRCA1/2 genes. In mutation carriers, family history characteristics may be similar to that seen in Caucasian patients. The oncology care setting may serve as an important point for identification and risk appropriate referral to genetic services for African American breast cancer patients.

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Full mutational analysis of the Chk2 gene in high risk Jewish Ashkenazi women. *E. Friedman[1,3], Y. Leitman[1,3], T. Distelman-Menachem[1,3], R. Gershoni-Baruch[2].* 1) Oncogenetics Unit, Inst. Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Institute of Genetics, Rambam Medical Center, Haifa, Israel; 3) Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel.

Germline mutations in BRCA1 and BRCA2 genes account for 20-40% of all familial breast cancer cases. The CHEK2 gene encodes a checkpoint kinase which is activated by ATM in response to DNA damage, and hence is a candidate gene for breast cancer susceptibility. The CHEK2*1100delC truncating mutation, originally described in a subset of Li-Fraumeni cases, was later reported in a subset of mostly European breast cancer families. The rate of the CHEK2*1100delC in the Ashkenazi population both in Israel and New York was reported to be 0.3%. To evaluate whether other variants of CHEK2 contribute to breast cancer predisposition in the Ashkenazi Jewish population we screened the entire coding region of CHEK2. Mutation analysis of the CHEK2 gene was performed using PCR followed by DGGE in high risk Jewish Ashkenazi women, none of whom was a carrier of the predominant Jewish mutations in BRCA1/BRCA2: 70 with breast cancer (average age of diagnosis 48.3 years, median 48.5) and 100 high risk asymptomatic individuals. Four previously described missense mutations were detected (R3W 1.2%, I157T 1.8%, R180C 0.6% and S428F 5%), one silent polymorphism (E84E 20.5%) and one novel missense mutation (Y424H 1.2%). Of the 10 carriers of S428F mutation, 8 were breast cancer patients (average age at diagnosis 50, range 41-57, median 51) and each had at least one other first degree family member affected with breast cancer, ovarian cancer was observed in a single pedigree. Of the remaining two healthy S428F carriers, one had a second degree relative affected with breast cancer and the other had a first degree relative affected with breast cancer and a second degree relative affected with ovarian cancer. The results of this study suggest that CHEK2 variants (other than 1100delC) may contribute to breast cancer susceptibility in Ashkenazi Jews.

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Hypoxia-inducible factor 1 alpha gene expression in prostate cancer measured by quantitative reverse-transcriptase polymerase chain reaction. *C. Pipinikas[1], S. Nair[1,2], R. Kirby[2], N. Carter[1], C. Fenske[1].* 1) Medical Genetics and; 2) Urology Department,St. George's Univ. London,SW17,UK.

INTRODUCTION There is an urgent need for accurate diagnosis of prostate cancer (CaP). Hypoxia is associated with cancer formation. Hypoxia-inducible factor-1 alpha (HIF-1)mediates activation of genes involved in cell survival and apoptosis. These normal cellular responses also give tumour cells a survival advantage. Quantitative RT-PCR (qRT-PCR)was used to measure HIF-1 expression levels and detect tumour cells in peripheral circulation. **METHODS** RNA was extracted in quadruplet from blood taken from 164 patients,divided into 4 diagnostic groups, based on clinicopathological information: no evidence of malignancy (NEOM), localised CaP (LCaP), metastatic CaP (MCaP) and radical prostatectomy (RP). qRT-PCR was carried out using HIF-1 specific primers and a LightCycler (Roche). **RESULTS** Highly significant differences in HIF-1 expression were found between all patient groups, with the exception of that between the NEOM and MCaP patient groups.

Patient Group	NEOM(N=36)	LCaP(N=67)	MCaP(N=27)	RP(N=34)
Mean (×10-4)	1.77	260.0	1.9	6.5
Median (×10-5)	6.2	81.0	6.9	23.0

DISCUSSION We have shown that HIF-1 together with qRT-PCR may be used for accurate diagnosis of CaP. Results indicate that hypoxia plays a role in the initial stages of CaP formation (150-fold increase in HIF-1 expression levels between NEOM and LCaP; $p<0.0001$). HIF-1 is down-regulated at advanced stages of the disease ($p<0.0001$ between LCaP and MCaP) suggesting the role of an alternative mechanism in the metastatic stages of CaP formation. High levels of HIF-1 were found patients following surgery (RP group). Of these,a high proportion were shown to have positive margins,highlighting the need for continued monitoring and additional therapy.

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Analysis of von Hippel-Lindau tumor suppressor gene mutations in clear cell RCC tumors using multiple platforms: an in-depth mutation screening strategy. *S. Lilleberg[1,2], K.B. Walters[2], Y. Shi[3], J.A. Durocher[2], W.-H. Chow[4], L.E. Moore[4], B. Zbar[5], G. Gerard[3], M.L. Nickerson[2].* 1) Discovery Services, Transgenomic, Omaha, NE; 2) Discovery Services, Transgenomic Gaithersburg, MD; 3) Research and Development, Transgenomic, Gaithersburg, MD; 4) Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 5) Laboratory of Immunobiology, NCI-Frederick, Frederick, MD.

Mutation of the VHL tumor suppressor gene has been implicated in the pathogenesis of clear cell renal carcinoma. A large case-control epidemiology study of kidney cancer is underway in Eastern Europe, which will, in part, examine how exposure history relates to the spectrum of mutations found in the VHL gene. In preparation for analysis of the VHL gene as part of this study, we rigorously examined Transgenomics independent mutation detection methodologies during analysis of the gene in a panel of patient tumor DNAs. Mutation analysis of the VHL protein coding region was performed on 28 patient tumors. Three methods of analysis were employed; denaturing high performance liquid chromatography (dHPLC), enzymatic digestion of PCR products with Surveyor Nuclease, and DNA sequencing. All platforms were able to detect point mutations, insertions, and deletions within the VHL gene. We found slight variation in sensitivity and specificity between the three individual approaches. Initial screening for mutations using Surveyor Nuclease and separation on a WAVE instrument followed by sequence characterization of variant positive amplicons allowed sequencing costs to be reduced considerably while maintaining 100% sensitivity. Integration of the 3 independent mutation detection platforms employed in this pilot study has the potential to expedite renal cancer research while reducing the cost of the analysis.

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Genome-wide Expression Analysis of Breast Cancer in African-Americans: New Insights into Genetic Mechanisms. *L. Baumbach, L. Gayol, M.E. Ahearn, L. Nathanson, B. Morel, R. Bookman, F. Moffat.* Miller School of Medicine, University of Miami, Miami, FL.

As is well known, breast cancer (BC) is the most common cancer in American women, and numerous studies have reported an excess incidence and mortality in AA women <50 yoa. To further investigate the genetic basis of these observed differences, we have conducted a novel pilot study analyzing genome-wide expression differences in self-matched breast cancer and normal tissue from African-American (AA) women: two sporadic cases and three with a family history of BC. Study recruitment criteria were either a sporadic case <50 years old/dx. with BC or a high-risk familial case of BC, and no radiation or chemotherapy prior to enrollment. Tumor and normal tissue samples were obtained fresh from surgery. Total mRNA from tumor tissue and the corresponding self-matched normal tissue were labeled with either Cy3 or Cy5 dye and then hybridized to the Agilent Whole Human Genome arrays. Dye-Swap labeling was completed for validation of hybridization data. These data were analyzed using Significance Analysis of Microarrays (SAM) with an FDR1%. Data from self-matched tumor and normal tissue samples were compared between familial and sporadic cases and between individual women in these two categories. The analysis yielded more than twice as many over expressed genes (2852) in the familial cases than in the sporadic cases. There were also 1988 under expressed statistically significant genes in the familial cases and about 200 less of these genes in the sporadic cases. About 1/8 of the reported over expressed genes and 1/3 of the reported under expressed genes were shared between the familial and sporadic groups. Importantly, there are 11 significant genes that are under expressed in sporadic and over expressed in familial cases, and 10 significant genes that are over expressed in sporadic and under expressed in familial cases (FDR1%). Additional analyses, including hierarchical clustering and real-time PCR are underway. This study should provide important information concerning the genetic differences between AA women with familial or sporadic BC.

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Hereditary Non-Polyposis Colon Cancer associated with a novel duplication mutation in the MSH2 gene encompassing exons 1 to 6. *B. Roa[1], L.H. Chin[1], B. Chong[1], T. Badger[2], P. Ward[1], H. Vance[3], A. Rajput[3], C. Le Vea[3], C. Farrell[3], M. Hegde[3].* 1) Medical Genetics Laboratories, Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Texas Tech University, Lubbock, Texas; 3) Roswell Park Cancer Institute, Buffalo, New York 14263.

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome is an autosomal dominantly inherited cancer syndrome characterized by malignancies of the colon and other sites. HNPCC results from germline mutations in DNA mismatch repair genes, primarily MSH2, MLH1 and to a lesser extent MSH6, PMS1 and PMS2. Identification of a germline mutation provides a molecular diagnosis in HNPCC patients. Sequence analysis can identify ~90% of germline mutations in the MSH2 and MLH1 genes. A proportion of HNPCC cases can be due to large gene rearrangements in the MLH1 and MSH2 genes which are refractory to PCR-based sequencing analysis. A founder mutation in MSH2 comprising of deletion of exons 1-6 has been reported to be one of the most frequent MSH2 mutations in North America. However, a reciprocal duplication has not been previously reported in HNPCC patients. We report on the identification of a novel duplication mutation encompassing exons 1 to 6 of the MSH2 gene. A Caucasian woman diagnosed with colorectal cancer at age 46 with a family history of colon cancer fulfilling the Amsterdam criteria was referred to our laboratory for MSH2 testing. Analysis on her colorectal tumor had shown high microsatellite instability on DNA testing, and absence of MSH2 and MSH6 expression on immunohistochemistry analysis. Sequencing did not identify a mutation in the MSH2 coding region. Analysis for gene rearrangements was performed using real time quantitative PCR, which showed duplication of MSH2 exons 1 to 6 with normal dosage of the remaining exons. This was corroborated by MLPA analysis. The exact endpoints have yet to be characterized to determine whether this novel duplication corresponds to the reciprocal recombination product of the MSH2 deletion founder mutation. Our data highlight the value of genomic rearrangement testing in the MSH2 and MLH1 genes as part of a comprehensive molecular diagnostic work-up for HNPCC.

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New DHPLC based method using qasimonomoprhic mononucleotide repeats and pentaplex PCR for rapid and accurate MSI analysis. *M. Ravnik-Glavac, G. Berginc, D. Glavac.* Department of Molecular Genetics, Institute of Pathology, Medical Faculty, Korytkova 2, 1000 Ljubljana, Slovenia.

Microsatellite instability (MSI) is a phenomenon characterized by small deletions or insertions within short tandem repeats in tumour DNA compared to matching normal DNA. MSI analysis is becoming more and more important for detection of herditary non-polyposis colorectal cancer as well as for sporadic primary colorectal tumours with MSI high phenotype. Set of two monucleotide and three dinucleotide microsatellite markers called Bethesda panel was proposed by National Cancer Institute Workshop in 1997 to provide uniform criteria for MSI analysis. A second consensus meeting was held at 2002 where guidelines were revised and exclusive use of mononucleotide instead of dinucleotide markers was proposed due to their quasimonomorphic nature. Use of five quasimonomorphic mononucleotide markers eliminates ultimate need for analysis of germline DNA corresponding to tumour DNA. Here we report a new method for MSI analysis using denaturating high performance liquid chromatography (DHPLC). We analysed 980 newly diagnosed colorectal tumours and 145 normal samples. Five quasimonomorphic mononucleotide microsatellite markers including BAT-25, BAT-26 were amplified in a single multiplex reaction and analysed using DHPLC and capillary electrophoresis. We have shown that tested markers are quasimonomorphic in Slovenian population with frequences of polymorphisms 0,07%, 1,4 for BAT-25, BAT-26 respectively. 71 new MSI-H tumours were identified among. Overall, we developed a high-throughput, robust, accurate and cost-effective method for detection of MSI-H tumours in only nine minutes using DHPLC. Method is suitable for large scale studies as well as for daily use with smaller numbers of samples.

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Linkage disequilibrium and founder *BRCA1* mutations: preliminary study in Ashkenazi Jews and other populations. *L.H.M. Pereira[1], M.A. Pineda[1], W.H. Rowe[1], L.R. Fonseca[1], M.H. Greene[2], K. Offit[3], N.A. Ellis[3], J. Zhang[1], A. Collins[4], J.P. Struewing[1,2].* 1) Lab of Population Genetics, &; 2) Clinical Genetics Branch, NCI, Bethesda, MD; 3) Memorial Sloan Kettering Cancer Center, New York, NY; 4) Human Genetics Division, University of Southampton, Southampton, UK.

We analyzed a dense set of SNPs in a 650kb region around *BRCA1*, a breast/ovarian susceptibility gene, in 5 populations, focusing on Ashkenazi Jews, a population with *BRCA1* founder mutations. We developed 143 SNP assays, of which 114 were polymorphic in at least 1 population. There were 84 intragenic (~ 1 SNP per kb) and 30 SNPs that flanked *BRCA1* (~ 1 SNP per 20kb). Subjects included 85 unrelated Ashkenazi Jews, 60 CEPH parents, and 48 each from African-Americans, Chinese-Americans and Mexican-Americans. Further, 6 unrelated 185delAG founder mutation carriers and their relatives were studied to define mutation-associated haplotypes from family data. We calculated pair-wise r^2 measures of LD using a modified version of LDSelect that does not assume Hardy-Weinberg equilibrium, estimated haplotypes using SNPHAP as implemented in Hapscope, determined haplotype block boundaries using Haploview, filtering SNPs with population-specific minor allele frequencies less than 5%, and calculated LDU length using LDMAP. The region analyzed shows long-range LD, falling primarily into 3 blocks among non-African populations. The entire *BRCA1* gene maps to a large block of at least 188kb that spans 95 markers (excluding a genome gap of arbitrary 100kb size). This block correlates completely with the largest LDSelect bin of SNPs correlated at $r^2 > 0.8$. The LDU map length among Ashkenazi Jews was 35% shorter than the CEPH map length. The 185delAG founder mutation occurs on the second-most frequent haplotype, representing 15% of chromosomes among Ashkenazi Jews, suggesting that a haplotype-based case-control association analysis would have been modestly enriched due to this founder mutation. Although in strong LD with the majority of markers studied as measured by D, owing to its relatively low frequency, the highest pair-wise r^2 for 185delAG was 0.04 with a SNP that mapped outside the large *BRCA1*-containing block, approx. 110kb downstream.

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Multicenter study to determine the frequency of germline mutations in MSH6 among various groups of patients with colon cancer. *M.L. Butz, S.N. Thibodeau, Colon Cancer Family Registry.* Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Current testing strategies to identify germline mutations in the MSH6 gene in families suspected of HNPCC are dependent on the sensitivity of IHC staining and MSI testing. To determine the frequency of germline mutations in MSH6 among different groups of patients having colon cancer, we utilized a subset of patients who participated in the Colon Cancer Family Registry. Direct DNA sequencing of the MSH6 gene was performed on 337 germline DNA samples provided by 2 main groups of subjects: 109 whose cancers had high MSI (MSI-H) and showed loss of MSH6 protein expression, either alone or in combination with other mismatch repair (MMR) proteins; and 228 whose tumors were either low MSI (MSI-L, n=95) or microsatellite stable (MSS, n=133) with normal IHC. The MSH6/MSI-H group consisted of cases that had loss of protein expression (by IHC) for either MSH6 alone (n=33), MSH2 and MSH6 (n=57), or MSH6 along with other MMR proteins (n=19). Of the MSH6-only cases, alterations were identified in 19 and in 16 (48%) they were pathogenic nonsense/ins/del mutations. Among the 57 MSH2/MSH6 cases, there were 3 nonsense (1 of unknown clinical significance - UCS) and 2 missense (UCS) alterations. Of the MSI-L cases, 1 missense mutation (UCS) and a 3 UTR deletion (UCS) were identified. Among the MSS cases, 4 missense (UCS) and 4 novel insertion/deletion mutations (UCS) were identified. As expected, pathogenic mutations were identified primarily among the group demonstrating loss of MSH6 only by IHC. The overall prevalence of 48%, however, is lower than anticipated. Other mechanisms need to be considered in these cases. Of interest, several likely pathogenic mutations were identified among patients showing loss of MSH2/MSH6. Thus, in such cases, testing MSH6 should be considered when an MSH2 mutation cannot be identified. Among the MSS/MSI-L group, a higher than anticipated frequency of missense mutations of UCS were identified. The significance of these alterations is under investigation. In summary, IHC and MSI analysis provides an efficient strategy for identifying MSH6 germline mutations.

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Expression phenotype of lymphoblastoid cell lines from carriers of the breast cancer-associated ATM mutation, 7271T>G (V2424G). *N. Waddell[1], J. Jonnalagadda[1], S. Grist[2], kConFab[3], M. Taylor[4], G. Suthers[2], K. Khanna[1], S. Grimmond[5], G. Chenevix-Trench[1].* 1) Queensland Inst Med Res, Brisbane, Australia; 2) South Australian Familial Cancer Service, Australia; 3) Peter MacCallum Cancer Institute, Melbourne, Australia; 4) University of Birmingham, UK; 5) Institute for Molecular Biosciences, Brisbane, Australia.

Heterozygous (ht) mutations in ATM (mutated in ataxia telangiectasia) have been associated with an elevated risk of breast cancer. We have reported one Australian breast cancer family in which ATM V2424G segregated with disease, and apparently acted as a dominant negative. To elucidate the consequences of this mutation, we expression profiled 5 wildtype (wt), 7 ht, and 3 homozygous (hom) LCLs from a Scottish and an Australian family using 19k oligo arrays, and found 60 significant differences between the genotypic groups. Cluster analysis revealed some discrete patterns of gene expression, including a large group of genes that are differentially expressed in hom and ht V2424G cells (compared to wt) and a small number differentially expressed in the hom cells (compared to wt and ht LCLs). We also evaluated the profiles of LCLs after exposure to ionizing radiation (IR) and identified a group of genes (over-represented in the DNA replication and cell cycle regulation pathways) that were differentially expressed in wt cells after IR, but not in hom or ht V2424G carriers. We validated the expression differences by RT-PCR in additional ht V2424G LCLs from one of two additional Australian breast cancer families that we have recently identified with the same mutation. We also found the same expression pattern in two other ht ATM LCLs with truncating mutations, suggesting a similar mechanism of action of V2424G and truncating mutations in ATM. We found no consistent cytotoxicity or abrogation of ATM kinase activity after IR in seven ht V2424G LCLs, compared to wt LCLs, but did find an increase in the number of chromosomal aberrations. These data suggest that the missense V2424G mutation, like truncating ATM mutations, act largely as a dominant negative in terms of the associated expression profiles and chromosomal aberrations.

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Does Nonsense-mediated mRNA Decay explain the Ovarian Cancer Cluster Region of the *BRCA2* Gene? *S. Mazoyer[1], D. DeSilva[2], O.M. Sinilnikova[3], D. Stoppa-Lyonnet[4], S.V. Tavtigian[2], M.D. Ware[1].* 1) Laboratoire de Génétique Moléculaire, Signalisation et Cancer UMR5201 CNRS, Université Claude Bernard Lyon I, 69373 Lyon cedex 08, France; 2) International Agency for Research on Cancer, 69372 Lyon cedex 08, France; 3) Plate-forme Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon / Centre Léon Bérard, 69373 Lyon cedex 08, France; 4) Service de Génétique Oncologique, Institut Curie, 75248 Paris cedex 05, France.

BRCA2 germline mutation carriers are at increased risk for breast and ovarian cancers. Mutations occurring in the Ovarian Cancer Cluster Region (OCCR) of *BRCA2* are linked to a higher risk of ovarian cancer and/or lower risk of breast cancer than mutations occurring outside this region. Most *BRCA2* germline mutations introduce premature termination codons (PTCs) that are likely to make their mRNAs targets of nonsense-mediated mRNA decay (NMD), a mechanism that detects and destroys PTC-bearing transcripts to prevent expression of truncated proteins. The PTC position in relation to downstream exon-exon junctions (EEJ) is an important factor in its recognition by NMD. Contradictory reports exist regarding the ability of NMD to recognize PTCs located far from the nearest 3 EEJ. The presence of the OCCR in the 4.9 kb exon 11 of *BRCA2* prompted us to investigate whether transcripts containing PTCs in this large exon avoided detection by NMD and whether this might contribute to the phenotypic difference associated with the OCCR. We observed a global reduction of 1.4 to 3.3-fold in the relative abundance of PTC-containing transcripts in lymphoblastoid cells of 18 truncating mutation carriers, regardless of the location of the PTC in the gene or its distance from the nearest 3 EEJ. We conclude that NMD is capable of recognizing PTCs up to 4.5 kb upstream of the nearest EEJ and that NMD-avoidance does not appear to be the biological mechanism behind the genotype-phenotype correlation associated with the OCCR.

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Presence of complete NF1 gene deletion is not frequently associated with the development of malignant peripheral nerve sheath tumours (MPNSTs). *M. Upadhyaya[1], E. Majounie[1], G. Spurlock[1], S. Griffiths[1], S. Huson[2], G. Evans[2], R. Ferner[3].* 1) Dept Medical Genetics, Univ Wales Col Medicine, Cardiff, United Kingdom; 2) Medical Genetics, St Mary's Hospital,Manchester, uk; 3) Clinical Neurosciences, Guy's Kings and St Thomas' School of Medicine, London, Uk.

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with an incidence of about 1 in 3,500. NF1 is characterised by a variety of benign and malignant lesions, including café-au-lait spots, neurofibromas, pilocytic astrocytomas and malignant peripheral nerve sheath tumours (MPNSTs). An increased incidence of malignant peripheral nerve sheath tumours (MPNSTs) in NF1 patients with microdeletions has been reported. We have studied DNA from 32 NF1 patients with MPNSTs for NF1 gene constitutional mutations to ascertain whether complete NF1 gene deletions are more frequently predisposed to the development of MPNSTs. Patients were clinically evaluated in London, Manchester,Oxford and Cardiff. Complete mutation analysis was conducted using SSCP,dHPLC,FISH, deletion breakpoint PCR and MLPA. Mutations were identified in a group of 30/32 patients.The age at diagnosis of MPNSTs ranged from 16 to 64 years and half of these patients have since died. Of the 32 patients, 20 had high grade MPNSTs, 9 had low grade MPNSTs while no details were given for three patients. Of the 30 mutations, nonsense mutations were identified in seven patients, microdeletions in 7 patients, micro-insertion in 3 cases, missense mutations in 3, splicing alterations in six and exonic/multi-exonic deletions identified in 3 patients. The common 1.5 Mb large deletion was only identified in one individual. The NF1 mutations identified were distributed evenly across the gene . There is no apparent relationship between the mutation type and the observed clinical severity. The overall mutation detection rate in our series was 94%.Thirteen of these sequence changes are novel. Our results indicate that NF1 patients with a variety of constitutional mutations may develop MPNSTs. Analysis of a larger panel of MPNSTs is clearly warranted to determine the risk related to microdeletion in MPNST formation.

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Global expression based classification of lymph node metastasis and extracapsular spread of oral tongue squamous cell carcinoma. *X. Zhou[1], S. Temam[2,3], B. Huang[1], M. Oh[4], N. Pungpravat[1], L. Mao[2], D.T. Wong[1,5].* 1) Dental Research Institute, School of Dentistry, University of California at Los Angeles, Los Angeles, CA; 2) Department of Thoracic/Head and Neck Medical Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX; 3) Department of Head and Neck Surgery, Institut Gustave-Roussy, Villejuif, France; 4) Department of Biostatistics, School of Public Health, University of California at Los Angeles, Los Angeles, CA; 5) Jonsson Comprehensive Cancer Center; Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA.

Tumor metastasis is the dominant cause of death in cancer patients, including oral tongue squamous cell carcinoma (SCC) patients. However, the molecular and cellular mechanisms underlying tumor metastasis are still elusive. The identification of molecular markers associated with the metastatic process would help to understand biological mechanisms and facilitate the development of therapeutic interventions for oral tongue cancer patients. We performed global gene expression profiling on 11 lymph node metastatic (pN+) and 14 non-metastatic negative (pN-) SCC samples of tongue, and the matching normal tissues. Using stringent statistical criteria (fold difference >2, and $p<0.003$), a signature set of 99 genes was generated that can classify the pN+ and pN- cases with > 90% accuracy. These analyses also generated a second gene list that can provide superb classification power for extracapsular spread (ECS). ECS is considered as the most reliable clinical predictor of poor treatment outcomes of metastatic squamous cell carcinoma of the head and neck to regional lymph nodes. Expression profiling capable of predicting ECS phenotype has not been reported previously, so these results have high substantive significance. Our results were further validated using real-time quantitative PCR, as well as publicly available microarray datasets of metastasis and non-metastasis head and neck cancers from the Gene Expression Omnibus (GEO) database.

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AGC repeat polymorphism in E2F-4 is associated with increased risk of gastric carcinoma. *L. Hu, Y. Yu, Y. Lu, W. Li, J. Ji, H. Xu, Q. Li, Z. Wang, Z. Zhu, B. Liu, Y. Lin, X. Kong.* Health Sci Ctr, Sibs, CAS, Shanghai, Shanghai, China.

E2F-4 protein is a member of the E2F family of transcription factors, playing an important role in DNA synthesis and cell cycle control. In its coding region, there is an AGC triplet repeat sequence, which has been reported to vary in some cancers. However, the characteristic of the AGC triplet repeat has never been detected systematically in gastric carcinoma. Here, we report four novel repeat variants in gastric carcinoma. Totally, 11 repeat variants and one SNP were detected in 20 of 85 tumors (23.53%), and among them 4 variants were detected in 9 of 11 MSI samples (81.82%). In healthy individuals, 3 variants were found in 7 out of 80 samples (8.75%). Overall, the AGC repeat polymorphisms occurred much more frequently in gastric carcinoma group than that in healthy (P=0.01, Odds Ratio was 3.21). The results demonstrated that people with AGC repeat variants had a higher risk of suffering gastric carcinoma than those without. Furthermore, immunohistochemical experiment revealed E2F-4 protein expression was obviously increased in diffuse-type gastric carcinoma than that in intestinal-type carcinoma and mixed carcinoma (P=0.017). All the results suggested E2F-4 AGC variants and increased expression maybe associated with predisposition and some clinical phenotype of gastric carcinoma.

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The role of genes related to DNA methylation process as modifiers of BRCA1/2 gene penetrance. *M.A. Caligo[1], C. Pepe[1], E. Sensi[2], P. Aretini[2], L. Guidugli[1], G. Cipollini[2], E. Falaschi[1], E. D'Andrea[3], M. Montagna[3], P. Radice[4], A. Viel[5], G. Bevilacqua[1].* 1) Department of Oncology, Division of Pathology,University Hospital and University of Pisa,Italy; 2) MGM,Pisa,Italy; 3) Department of Oncology and Surgical Sciences, Oncology Section, Azienda Ospedaliera, Padua, Italy; IST-Genova c/o Department of Oncology and Surgical Sciences,Azienda Ospedaliera, Padua, Italy; 4) National Cancer Institute, Milan, Italy; FIRC Institute of Molecular Oncology, Milan, Italy; 5) Centro di Riferimento Oncologico IRCCS, Aviano Italy.

Significant variations in the level of risk associated with a mutation of BRCA1/2 genes have been highlighted, suggesting the existence of modifiers of gene penetrance. DNA methylation is an important mechanism of transcriptional regulation. Polymorphisms in enzymes involved in SAM (S-adenosylmethionine) metabolism, such as MTHFR (methylene tetrahydrofolate-reductase), MS (methionine synthase) and CBS (cystathionine beta-synthase) are good candidates for affecting the pattern of DNA methylation.The aim of the study is to investigate the role of genes related to DNA methylation process as possible modifiers of the BRCA1/2 gene penetrance. We genotyped 348 affected and 115 unaffected BRCA1/2 mutation carriers (of which 133 affected and 32 unaffected BRCA1-carriers and 215 affected and 83 unaffected BRCA2-carriers) and a control group. Four polymorphisms have been considered: C677T and A1298C for MTHFR gene, A2756G for MS gene and Ins68bp for CBS gene.The analysis has been performed by means of PCR-RFLPs. A Cox regression analysis on age of breast cancer onset showed that the BRCA1 carriers with the MTHFR gene 677T allele exhibited a lower age of onset of the breast disease (P=0,04 HR=1.73).The HR and the confidence limits were adjusted by provenience group and by year of birth (before and after 1960).Moreover the analyses were corrected for the familial aggregation.No effect on BRCA2 gene penetrance was observed.A MSP(Methylation-Specific PCR) analysis of the promoter region of BRCA1 gene in tumor samples will allow to test the effect of a low apport of the methyl-group donor SAM on gene inactivation.

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Induction of renal tumourigenesis with elevated levels of somatic LOH in *Tsc1*$^{+/-}$ mice on a *Blm*-deficient background. *J.P. Cheadle, C. Wilson, S. Idziaszczyk, J. Colley, V. Humphreys, C. Guy, J. Maynard, J.R. Sampson.*
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A Bloom (RecQ) deficient mouse model (*Blm*$^{m3/m3}$) has been shown to induce colorectal tumourigenesis when crossed with *Apc*$^{Min/+}$ mice (Luo *et al.* 2000). Here, we investigated whether *Blm*$^{m3/m3}$ mice could induce tumourigenesis in extra-colonic tissues by crossing with *Tsc1*$^{+/-}$ mice that are predisposed to renal cystadenomas and carcinomas (Wilson *et al.* 2005). Genotyping of offspring from *Tsc1*$^{+/-}$ *Blm*$^{+/m3}$ intercrosses showed that a ~24% excess of *Tsc1*$^{+/-}$ mice died before weaning (P= 0.016), in agreement with our previous study (Wilson *et al.* 2005). *Tsc1*$^{+/-}$ *Blm*$^{m3/m3}$ mice had significantly more macroscopic and microscopic renal lesions at 3-6 months compared to *Tsc1*$^{+/-}$ *Blm*$^{+/m3}$ mice (p=0.0003 and p=0.0203, respectively), and showed significantly increased levels of somatic LOH of the wild type *Tsc1* allele, as compared to *Tsc1*$^{+/-}$ *Blm*$^{+/+}$ mice (p<0.0001). *Tsc1*$^{+/-}$ *Blm*$^{+/m3}$ mice did not show significantly more renal lesions compared to *Tsc1*$^{+/-}$ *Blm*$^{+/+}$ animals; however, their lesions still showed significantly increased levels of somatic LOH (p=0.028). Renal lesions on a *Blm*-deficient background stained positively with anti-Phospho-S6 Ribosomal protein (Ser 240/244) indicating that these lesions develop through the normal pathway of *Tsc*-associated tumourigenesis. This work demonstrates the utility of the *Blm*-deficient mice for inducing renal tumourigenesis and the high levels (~82%) of LOH in the resultant tumours will help facilitate mapping of loci involved in tumour progression.

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The contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer. *S.J. Ramus[1], P. Harrington[2], R.A. DiCioccio[3], B.A. Werness[4], K. Garlinghouse-Jones[5], I. Oakley-Girvan[5], C. Pye[2], I.J. Jacobs[1], A.S. Whittemore[5], B.A.J. Ponder[2], P.D. Pharoah[2], M.S. Piver[3], S.A. Gayther[1].* 1) Department of Gynaecological Oncology, University College London, UK; 2) Department of Oncology, University of Cambridge, UK; 3) Gilda Radner Registry at Roswell Park Cancer Institute, Buffalo, NY, USA; 4) Department of Pathology, Inova Fair Oaks Hospital, Fairfax, VA, USA; 5) Department of Health Research and Policy, Stanford University School of Medicine,CA, USA.

Three hundred families, from the UKCCCR and Gilda Radner ovarian cancer registries, containing at least two confirmed cases of epithelial ovarian cancer (EOC) in first-degree relatives were screened for coding mutations in the BRCA1 and BRCA2 genes and genomic deletion/rearrangement mutations in BRCA1. An affected individual from each family was first screened by SSCP/HA analysis. Abnormal variants were sequenced to identify the causative nucleotide change. Families in which no deleterious mutation was identified (174 families) were further analysed for genomic rearrangements of BRCA1 using MLPA. Functional BRCA1 mutations were identified in 111 families (37%), of which 10 were detected by MLPA; thus 9% of all BRCA1 mutations were large rearrangements. Of these, 5 were the same exon 13 amplification (a UK founder mutation). The remaining 5 rearrangements were previously unreported deletions of exon 2, exons 3-16, exons 8-13, exons 15-20, and exons 21-24. Functional BRCA2 mutations were identified in 25 families (8%). BRCA1/2 mutation prevalence correlated with the extent of ovarian and breast cancer in families. Of families with >2 EOC cases and at least one breast cancer case under 60 years, 86% had a BRCA1/2 mutation. Mutation prevelence was significantly less in families containing no breast cancer. Of 156 families containing 2 ovarian cancer cases, only 28% had an identifiable mutation compared to 64% of 58 families containing 3 or more EOC cases. These data indicate that BRCA1 and BRCA2 are the major EOC susceptibility genes; but that other susceptibility genes may also exist.

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Multiplex PCR as sensitive method for *BRCA* founder mutation detection in formalin-fixed and paraffin-embedded (FFPE) tissue. *M.A. Adank[1,4], E. Brogi[2], F. Bogomolniy[3], E.A. Wadsworth[1], K.J. Lafaro[1], C. Yee[1], E.J. Meijers-Heijboer[4], N.D. Kauff[1], J. Boyd[1,3], K. Offit[1].* 1) Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center (MSKCC), New York, NY; 2) Dept. of Pathology, MSKCC, New York, NY; 3) Dept. of Surgery, MSKCC, New York, NY; 4) Dept. of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

Statement of purpose: To determine the sensitivity and reproducibility of *BRCA1* and *BRCA2* founder mutation analysis in formalin-fixed and paraffin-embedded (FFPE) tissue so it can be offered in a clinical setting. **Methods:** DNA extracted from 161 coded, morphologically normal FFPE samples (from women who were previously genotyped on lymphocyte derived DNA by direct sequencing) were analyzed simultaneously for the presence of one of the three common Ashkenazi founder mutations in *BRCA1* (5382insC and 185delAG) and *BRCA2* (6174delT) with multiplex PCR followed by electrophoresis on denaturing polyacrylamide gels. **Results:** A total of 120 *BRCA* founder mutations (39 *BRCA2*6174delT*, 24 *BRCA1*5382insC* and 57 *BRCA1*185delAG*) and 40 wild type samples were detected, including 25 samples over 10 years old. Only one of the 161 morphologically normal FFPE tissue samples was excluded due to PCR-failure. There was a 100% sensitivity and concordance between DNA results of the 160 samples derived from archival FFPE tissue and fresh lymphocytes. **Conclusions:** The multiplex PCR method had 100% sensitivity in detecting 120 Ashkenazi *BRCA* founder mutations in a time-efficient way. This is the largest described comparative study on *BRCA* testing of FFPE tissue; prior studies have shown highly variable sensitivity of this approach. This method has high potential for clinical testing in dominantly inherited breast and ovarian families where the only affected women are deceased. By testing them it allows clarification of uninformative negative interpretations for a subset of unaffected probands, mitigating the need for intensified surveillance and/or risk-reducing surgery. This method may also be useful in other populations were founder mutations are the predominant mutations in *BRCA1* and *BRCA2*.

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A high frequency of germline NF2 mutations detected with complementary mutation screening techniques. *A. Rohlin[1], J. Wahlström[1], L. Wiklund[2], M. Nordling[1].* 1) Clinical Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden; 2) Dept of Neurology, University Hospital, Uppsala, Sweden.

Neurofibromatosis type 2 (NF2) is an autosomal, dominantly inherited tumor syndrome with high morbidity and mortality caused by mutations in the NF2 gene. Bilateral vestibular schwannomas are the most frequent manifestations of the disease, other manifestations are; central and peripheral nervous system schwannomas, cereberal meningiomas and ocular abnormalities. In about half of the patients the mutation occurs as a new-mutation i.e. the family does not show any heredity for NF2.

Since 2000 we provide genetic and clinical investigations for Swedish NF2 patients. Initially we use DNA sequence determination and Multiplex Ligation-dependent Probe Amplification (MLPA), to detect sequence variations and large deletions/duplications in the NF2 gene. In new-mutation cases, mosaics (i.e. the mutations are only present in a group or population of cells) are frequently encountered. If no mutation is found using initial DNA-sequence determination or MLPA the sample is analyzed with DHPLC (Wave system equipped with the HSX fluorescent detector) and /or SSCP to enhance detection of low-frequency mutation alleles in mosaic samples. To detect mutations causing aberrant splicing and to verify MLPA findings mRNA (cDNA) is analysed. Total loss of NF2 transcripts is also investigated using coding SNP polymorphism on cDNA. Mutations where detected in 20/31 (64%) of patients with verified NF2 disease, including two mutations not reported earlier. A genotype-phenotype correlation was observed in a mosaic case and in a patient carrying a splice-site mutation, both giving rise to milder disease.

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Mutation screening in Juvenile Polyposis Syndrome. *R.E. Pyatt[1], R. Pilarski[2], T.W. Prior[1].* 1) Dept Pathology, Ohio State University, Columbus, OH; 2) Department of Internal Medicine, Division of Human Genetics, Ohio State University, Columbus, OH.

Juvenile polyposis syndrome (JPS) is an autosomal dominant cancer predisposition syndrome characterized by congenital anomalies, hamartomatous polyps in the colorectal and upper gastrointestinal regions, and the development of tumors in these tissues. The clinical diagnosis of JPS is often difficult due to the phenotypic overlap with other syndromes featuring hamartomatous polyps. Recently germline mutations have been identified in SMAD4 and BMPR1A, two members of the TGF- superfamily, which has aided in presymptomatic genetic testing. In this study, we describe the results from three years of molecular diagnostic screening for germline mutations in these genes. Seventy unrelated individuals referred to our lab for JPS testing were examined by direct sequence analysis of all coding regions and exon-intron boundaries in both genes. Mutations were identified in thirty percent of cases, with eleven percent in BMPR1A and eighteen percent in SMAD4. All mutation positive individuals were negative for cancer at the time of testing and a single case of pulmonary valve stenosis was the only congenital anomaly reported. A majority of mutations identified in both genes were novel including the first splice site alteration described in SMAD4. Based on the limited number of exons in either gene, low polymorphism frequency, and high frequency of frameshift or nonsense mutations identified, direct sequence analysis is an suitable methodology for mutation screening in JPS. The large number of unique mutations, the absence of mutational hotspots, and similar mutation frequencies between genes mandate that all coding regions and exon-intron boundaries must be examined.

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Mutational screening of MLH1, MSH2, and APC genes in Chilean patients with hereditary colorectal cancer. *K. ALVAREZ[1], M. DE LA FUENTE[2], A. LETELIER[2], F. LEON[1], F. BELLOLIO[1], F. LOPEZ-KOSTNER[1], M.P. CARVALLO[2].* 1) Departamento de Cirugía Digestiva, Facultad de Medicina, P. Universidad Católica de Chile, Santiago, Chile; 2) Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

Two hereditary variants have been described in Colorectal Cancer: HNPCC (hereditary non polyposis colorectal cancer) caused by mutations at the MLH1 and MSH2 genes, and FAP (familial adenomatous polyposis) caused by mutations at the APC gene. In Chile there is an increase in the incidence of colorectal cancer in the last years. For this reason we proposed to study the MLH1 and MSH2 genes in HNPCC families, and the APC gene in FAP families. Four HNPCC families and seven FAP families were selected by standard criteria. All exons and intron-exon boundaries of the three genes were analyzed through Single Strand Conformation Polymorphism, Protein Truncation Test, and sequencing. In the HNPCC families we found one allelic variant, non described previously, IVS15+3A>T, and one mutation c.504insA in exon 6, both in the MLH1 gene. The c.504insA frameshift mutation causes a premature termination at the codon 171. In FAP families we found four mutations in the APC gene: c.790C>T (Q264X) in exon 7 in one family, c.3927_3931delAAAGA in exon 15 in two families, c.3941_3942delG in exon 15 in one family and c.3920T>A (I1307K) in exon 15, previously described for Ashkenazi-Jewish families. The first three mutations lead to premature translation termination, being the c.3927_3931delAAAGA, the most frequent mutation described in FAP patients. Mutations in IVS 15+3A>T in MLH1, c.790C>T in exon 7 of APC, and c.3941_3942delG in exon 15 of APC constitute novel germline mutations. This is the first mutational report in Chilean HNPCC and FAP patients. These three novel mutations contribute to the world genetic database. FONDECYT 1040827.

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Screening of the ATM gene, and CHEK2 1100delC in 40 Chilean families with high risk to breast cancer. *M.P Carvallo[1], T. Tapia[1], A. Sánchez[1], C. Alvarez[1], S. Smalley[1], A. Letelier[1], M.C. Vallejos[1], M. Alvarez[2].* 1) Facultad de Ciencias Biologicas, P. Universidad Catolica de Chile, Santiago, Chile; 2) Centro de Cáncer, Facultad de Medicina, P. Universidad Católica de Chile, Santiago, Chile.

The major genetic factor for breast cancer high risk in families, is the presence of mutations in the BRCA1 or the BRCA2 gene. Several studies have shown that the prevalence of mutations among families is variable, and depends on the ethnic origin of the population analyzed. In a previous study in 54 Chilean families, we found that only 20% presented mutations in one of these genes. It has been mentioned that other low penetrance genes, like ATM and CHEK2, that are components of the ATM-dependent signalling pathway, as BRCA1 and BRCA2, may explain part of the hereditary predisposition to breast cancer. For ATM it has been shown that female heterozygous carriers for mutations in this gene have an increased risk of breast cancer. For CHEK2, one mutation 1100delC has been involved in predisposition to breast cancer for some populations. We performed a screening of the ATM gene in 40 non BRCA1 or BRCA2 patients from our previous study. We screened the 62 exons and intron-exon boundaries from the ATM gene for mutations, through SSCP and sequencing. We found five intronic polymorphisms (homozygous or heterozygous), with no significant differences in frequency between cases and controls. Three heterozygous missense mutations: c5557G>A (D1853N), c2572T>C (F858L), c5558A>T (D1853V) were also found. No significant difference was found in the frequency of the variant allele c2572C (F858L) between 40 cases and 50 controls. However the c5557A (D1853N) variant allele was significantly associated with an increased risk of developing breast cancer (allele A vs allele G OR 4.8, 95% CI 2.74-8.39 , P< 0.05) The other missense mutation c5558A>T was found in 1/40 patient and not present in 50 controls. These results provide evidence that the c5557A (D1853N) variant allele might be associate with familial breast cancer risk in Chilean families. The CHEK21100delC mutation was not found in patients neither in 200 controls, from the Chilean population. FONDECYT 1040779.

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The High Cumulative Incidence of Cancer in Individuals with Blooms Syndrome Does Not Vary by Sex, Ethnicity, or Mutation Type. *A. Zauber[1], M. Sanz[2], W. Shi[1], J.L. German[3].* 1) Dept Epidemology/Biostatistics, Mem Sloan Kettering Cancer Ctr, New York, NY; 2) Molloy College Kellenberg Hall, Rm105 1000 Hempstead Ave POBox 5002 Rockville Centre, NY 11571-5002; 3) Weill Cornell Medical College 1300 York Ave New York, NY 10021.

Blooms syndrome (BS) is a rare recessively transmitted form of proportional dwarfism that predisposes to many types of cancer. The gene mutated in BS encodes a large nuclear protein known to have DNA helicase activity. 64 BS-causing mutations have been identified and characterized at the molecular level; they can be classified as to whether the peptide they encode enters/does not enter the nucleus. The product of one mutant allele, blm^{Ash}, an allele present in ~1% of Ashkenazi Jews and in all Ashkenazi Jews with BS, is among many that fail to enter the nucleus; in contrast, the products of 11 missense mutations and the 1 splice-site mutation examined so far, though demonstrably lacking in helicase activity, do assume a roughly normal (nuclear)distribution. The features and clinical courses of 238 individuals with BS, along with certain features of the causative mutations, have been entered into a recently developed ACCESS database,the Bloom's Syndrome Registry Database. From this database we determined (i) the cumulative incidence of cancer (CIC) and also whether the incidence of cancer varies with respect to (ii) sex, (iii) ethnicity, and, or, (iv) mutation type. The CIC was 10% by age 10, 23% by age 20, 45% by age 30, and 82% by age 40. (The cumulative mortality was also high, 74% by age 40, with most deaths due to cancer.) CIC was similar for males (49%) and females (40%) by age 30 (hazard ratio [HR]=1.0 and 95% Confidence Interval [CI] 0.7-1.5) and for Jews (46%) and non-Jews (43%) by age 30 (HR=1.1; CI 0.7-1.7). Also CIC was similar with respect to whether the mutant proteins were/were not nuclear localizing. Thus, different classes of known BS-causing mutations are implicated in BS's extraordinarily great risk of cancer, and that risk is no greater in one sex than in the other, nor in Jews than in non-Jews.

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Genetic Analysis of Candidate Modifier Genes in BRCA1 and BRCA2 Mutation Carriers. *D.J. Hughes[1], I. Coupier[2], L. Barjhoux[1], V. Gaborieau[1], G. Lenoir[3], P. Brennan[1], D. Stoppa-Lyonnet[2], O.M. Sinilnikova[1,4].* 1) Unit of Genetic Epidemiology, International Agency for Research on Cancer, 150 cours Albert Thomas, 69008 Lyon, France; 2) Service de Génétique Oncologique, Institut Curie, 75248 Paris, France; 3) Institut Gustave Roussy, 94805 Villejuif, France; 4) Centre Léon Bérard, 69373 Lyon, France.

Characterisation of deleterious germline alterations in the BRCA1 and BRCA2 genes has provided a genetic approach to identification of high-risk women for breast and ovarian cancer. Despite the high overall lifetime risk of these cancers among mutation carriers, there is marked variation in risk both between and within families. However, the basis of these strong inter-individual risk differences is very poorly understood and may be partly explained by modifier genes that influence mutation penetrance. We are using association studies to assess the influence of biologically plausible candidate loci on the penetrance of BRCA1/2 mutations. An understanding of these modifying effects should help refine individual risk estimates and is also expected to be an efficient method to identify further susceptibility alleles in general, as variants within low penetrance predisposition genes are likely to have a larger risk modification effect in BRCA1/2 mutation carriers. Several potential genetic modifiers in BRCA1/2 carriers have been reported including a polymorphism in the 5UTR region of the RAD51 gene, and poly-glutamine coding variation in the AIB1 gene. We have analyzed these variants in our sample set of around 1200 BRCA1/2 mutation carriers. We have focused on variants from several DNA repair genes, as this seems the major pathway in which BRCA1 and BRCA2 operate, and from genes whose products interact with the BRCA proteins. A more extensive genetic analysis of two of these latter genes, BARD1 and ACCA which are located in the same genomic region as BRCA1, has also been undertaken. For none of these genetic variants did we find any evidence of a major breast cancer modifying effect. We did see some effects for ovarian cancer but believe this could easily represent false positives, due to the low number of these cases in our sample set.

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Neurofibromas from NF1 patients retain neurofibromin expression. *T. Tucker, CJ. Brown, JM. Friedman.* Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada.

Neurofibromatosis 1 (NF1) is an autosomal dominant disease affecting 1 in 3500 people. Neurofibromas are the hallmark of NF1 and are classically divided into four groups based on occurrence and clinical behaviour: cutaneous, subcutaneous, nodular plexiform and diffuse plexiform. These benign tumours develop from peripheral nerves and are comprised largely of Schwann cells but may contain numerous fibroblasts, perineural cells, endothelial cells and mast cells. All classes of neurofibromas are generally believed to develop in accordance with Knudsons two-hit hypothesis, and, as expected, loss of heterozygosity (LOH) at the *NF1* locus has been demonstrated. However, only 30% to 50% of neurofibromas analysed have shown LOH, even in tumour-derived $S100^+$ (presumably Schwann cell) lines. The low percentage of observed *NF1* LOH is usually attributed to technical limitations of the LOH assay, which is not capable of identifying point mutations or epigenetic mechanisms that may inactivate the normal *NF1* allele, or to contamination of the neoplastic cells by non-neoplastic stromal. We have used dual-label immunofluorescence to demonstrate the expression of neurofibromin, the protein product of the *NF1* gene, in tissue from nodular and diffuse plexiform neurofibromas from individuals with NF1. Diffuse plexiform neurofibromas demonstrated clusters of $S100^+$ (presumably Schwann) cells interspersed with $S100^-$ cells. An average of 63% of the $S100^+$ cells also expressed neurofibromin. Nodular plexiform neurofibromas showed a more even distribution of $S100^+$ cells, the majority (73%) of which also expressed neurofibromin. These findings are not consistent with an obligate requirement of complete loss of NF1 function as a prerequisite for neurofibroma development. Rather, our observations, which join an increasing body of evidence in other inherited tumour predisposition syndromes, suggest that some neurofibromas may develop in haploinsufficient tissues without the need for a second hit at the tumour suppressor locus. Examining neurofibromin expression in each type of neurofibroma may provide further information on the pathogenesis of these clinically distinct tumours.

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Identification and characterization of novel SNPs in CHEK2 in Ashkenazi Jewish men with prostate cancer.

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To identify variants (q > 0.01), we re-sequenced all exons and intron-exon boundaries of CHEK2 in 75 Ashkenazi Jewish (AJ) individuals with prostate, breast or no cancer (n = 25 each). We identified 7 coding SNPs (5 are novel) that changed the amino acid sequence, resulting in R3W, E394F, Y424H, S428F, D438Y, P509S and P509L. We determined their frequency in probands from 77 AJ families collected by ICPCG members where 2 men were affected by prostate cancer and 1 affected man provided a DNA sample. Only 1 variant, Y424H was identified in more than 2 affecteds. Exon 11 was screened in 10 additional families for a total of 87 families with at least one affected genotyped. The Y424H variant occurred in 9 affecteds in 4 different families. In 1 family, all 3 affecteds had the variant. In another, 4 of the 5 affecteds carried the Y424H variant. For the other 2 families, only 1 affected out of 2 or 3 affecteds had this variant. Bioinformatic analysis showed that Y424H is a radical missense substitution that falls at a position that is invariant in vertebrate CHEK2 orthologs. Both SIFT and Align/GV-GD predict that this is a loss of function mutation. However, Y424H frequency was 8/709 in prevalent cases and 5/545 in controls (OR 1.23, 95%CI: 0.35-4.82, P =.79). These results suggest that CHEK2 has a minor role, if any, in prostate cancer susceptibility in the AJ population. However, for = 0.05 and a power of 80%, a study of > 9000 cases and > 9000 controls will be required to rule out an OR of 1.5 in association with Y424H.

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Impact of the MDM2 SNP309 and TP53 Arg72Pro polymorphism on age of tumor onset in Li Fraumeni syndrome. *G. Bougeard[1,2], S. Baert-Desurmont[1,2], I. Tournier[1], S. Frerot[2], C. Martin[2], D. Stoppa-Lyonnet[3], C. Bonaiti[4], T. Frebourg[1,2].* 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Department of Genetics, Rouen University Hospital, France; 3) Department of Genetics, Institut Curie, France; 4) Inserm U535, Villejuif, France.

The Li-Fraumeni syndrome (LFS), which results from germline mutations of the TP53 gene, represents one of the most devastating genetic predispositions to cancer characterized by a wide spectrum of early-onset malignancies. Recently, the SNP309 (T>G variation) in the MDM2 gene, encoding a negative regulator of TP53, was shown to be associated with accelerated tumor formation in TP53 mutation carriers. The impact of the common TP53 Arg72Pro polymorphism on tumorigenesis remains controversial. We have therefore investigated the effect on tumorigenesis of these 2 polymorphisms in 61 germline TP53 mutation carriers. The mean age of tumor onset in patients homozygous for the MDMD2 SNP309 T allele was 29.9 years whereas it was 19.6 years in G allele carriers. This difference was significant ($p<0.05$). Patients homozygous for the TP53 Pro allele had a mean age of tumor onset of 34.4 years, which was significantly different from that observed in Arg allele carriers (21.5 years, $p<0.05$). We observed a cumulative effect of both polymorphisms since the mean ages of tumor onset in patients, with the MDM2 T/T and TP53 Pro/Pro genotype, and in patients, carrying at least one MDM2 G allele and at least one TP53 Arg allele, were clearly different (43 years versus 16.9 years, $p<0.02$).We did not detect any effect of these polymorphisms on the tumour type. Neither the MDM2 nor the TP53 polymorphism could explain in our series the incomplete penetrance of germline TP53 mutations observed in males. Our results confirm the impact on the age of tumour onset, in germline TP53 mutation carriers, of the MDM2 SNP309 G and TP53 72 Arg polymorphism, which represent the first modifier genetic factors identified in mendelian forms of cancer. However, detection of these polymorphisms should not modify the genetic counselling and clinical follow-up of LFS families with germline TP53 mutations.

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Population-based estimates of breast cancer risks associated with the *ATM* gene variants 7271TG and IVS10-6TG from the Breast Cancer Family Registry. *S. Teraoka[1], J.L. Bernstein[2], M.C. Southey[3], M.A. Jenkins[3], E.M. John[4], J. Knight[5], I.L. Andrulis[5], R. Lapinski[6], G. Chenevix-Trench[7], A. Wolitzer[2], E.R. Olson[1], A.S. Whittemore[8], D. Seminara[9], J.L. Hopper[3], P. Concannon[1], Breast Cancer Family Registry.* 1) Benaroya Research Inst, Seattle, WA; 2) Memorial Sloan Kettering Cancer Ctr, NY, NY; 3) University of Melbourne, Melbourne, AUS; 4) Northern California Cancer Ctr, Fremont, CA; 5) Samuel Lunenfeld Research Inst., Toronto, CAN; 6) Mt. Sinai School of Medicine, NY, NY; 7) Queensland Inst. of Medical Research, Brisbane, AUS; 8) Stanford University School of Medicine, Stanford, CA; 9) National Cancer Inst., Bethesda, MD.

The *ATM* gene variants segregating in ataxia-telangiectasia families are associated with an increased breast cancer risk, but the contribution of specific variants has been difficult to estimate. Previous small studies suggested that two functional variants, 7271TG and IVS10-6TG, are associated with increased if not high risk. Using population-based samples we found that 7 of 3,743 cases (0.2%) and 0 of 1,268 controls were heterozygous for the 7271TG allele (P = 0.1). In cases, the allele was more prevalent in women who had an affected mother (odds ratio (OR) = 5.5, 95% confidence interval (CI) 1.2-26; P = 0.04) or delayed child-bearing (OR = 5.1, 95% CI 1.0-25.6; P = 0.05). Based on breast cancer history of first- and second-degree relatives of carrier cases, the estimated cumulative breast cancer risk to age 70 years (penetrance) was 52% (95% CI 23-86; hazard ratio = 8.6, 95% CI 3.1-23.6; P 0.001). In contrast, 13 of 3,757 cases (0.3%) and 10 of 1,268 controls (0.8%) were heterozygous for the IVS10-6TG allele (OR=0.44, 95% CI = 0.19-1.0; P = 0.05). This suggests that the rare *ATM* 7271TG variant is associated with a substantially elevated breast cancer risk, but the more common IVS10-6TG variant is not associated with increased risk. Since 7271TG is only one of many rare *ATM* variants predicted to have deleterious consequences, effective means of identifying and grouping these variants will be essential to accurately assess the contribution of *ATM* variants to individual risk and population burden of breast cancer.

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Evaluation of pre-test probability models in identifying MLH1 and MSH2 mutations in HNPCC: A retrospective study. *K.W. Jasperson, K. Lowstuter, J.N. Weitzel.* Clinical Cancer Genetics, City of Hope , Duarte, CA.

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant syndrome characterized by a susceptibility to colorectal, endometrial, and other cancers. The accuracy of available models for predicting mismatch repair (MMR) mutation probability is unknown. The purpose of this study is to evaluate the Wijnen et al. (1998) pre-test probability model and the Myriad Genetics Laboratories, Inc. prevalence table (2004) at predicting the likelihood of a mutation in individuals undergoing genetic testing for HNPCC. A retrospective analysis was performed in 49 patients that were enrolled in an IRB-approved hereditary cancer registry. Patients were previously screened for germline mutations in MLH1 and MSH2. Sensitivity, specificity, and negative and positive predictive values were calculated for the Amsterdam criteria I and II, modified Bethesda guidelines, Wijnen model, and Myriad table. In addition, receiver operator characteristic (ROC) curves were calculated for the Wijnen model and Myriad table. Of the 49 individuals, 18 were found to harbor a germline mutation in MLH1 or MSH2. Using a 10.0% MMR gene mutation probability threshold, the Wijnen model and Myriad table performed poorly with both low sensitivities (55.6% and 60.0% respectively) and specificities (54.8% and 23.8% respectively). As expected, the Bethesda guidelines showed a high sensitivity (94.4%) and low specificity (12.9%), while the Amsterdam criteria I and II together had a sensitivity of (38.9%) and specificity of (71.0%). In addition, the calculated pre-test probabilities from the Wijnen model and Myriad table inadequately correlated with mutation prevelance, which is shown by the small areas underneath their corresponding ROC curves (0.616 and 0.400 respectively). The results of this study demonstrate that the neither the Wijnen model nor the Myriad table are sensitive or specific enough to be useful in a clinical setting at prediciting MMR mutations in individuals suspected of having HNPCC.

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c-Myc over-expression of Ramos Burkitt's lymphoma cell line predisposes to oxidative stress and to free radicals induced damages *in vitro*. *M. Habel[1,2], D. Jung[1,2].* 1) Research & Development, Héma-Québec, Sainte-Foy, Québec, Canada; 2) Laval University, Sainte-Foy, Québec, Canada.

Burkitt's lymphoma is an aggressive B-cell neoplasm resulting from deregulated *c-myc* expression. We previously showed that proliferation of BL cell lines such as Ramos is markedly reduced by iron . Furthermore, this effect is mediated by a cell cycle arrest in G_2/M. It has been shown that iron induces expression of *c-myc* which regulates genes involved in iron metabolism. Over expression of *c-myc* and transient enhancement of its expression by iron could increase the expression of genes involved in iron incorporation, which could lead to an accumulation of intracellular free iron. Indeed, intracellular free iron catalyzes the formation of highly reactive compounds such as hydroxyl radicals and nitric oxide that damages macromolecular components of cells, resulting in apoptosis. Here, we have investigated whether cells with a high basal level of c-Myc were more likely to accumulate free iron and the possible involvement of free radicals in the response of Ramos cells to iron. Our results suggest that in Ramos cells, where c-Myc is expressed at a high level, *H-ferritin* expression is down-regulated, transferrin receptor expression is increased and ferritin translation is inhibited. These modifications resulting from the strong basal expression of c-Myc, and amplified by iron addition, lead to a disruption in homeostasis and consequently to growth arrest mainly by increasing the intracellular levels of peroxide/peroxynitrite and NO. Moreover, the addition of free radicals scavengers neutralized the iron-induced free radicals accumulation in Ramos cells. Additional experiments provided support for the involvement of ROS in the G_2/M blockade, whereas growth inhibition appear to be primarily mediated by NO. Furthermore, addition of an NO donor or H_2O_2 to Ramos cells generated effects which partially mimicked those induced by iron. Collectively, our results suggest that cells with high expression of c-Myc are predisposed to homeostasis disruption leading to oxidative stress and that free radicals act as effectors in the iron specific growth inhibition of BL cells observed *in vitro*.

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Immortalization of normal esophageal epithelial cells by human telomerase reverse transcriptase or human papilloma virus gene E6E7: sequential cytogenetic and molecular genetic characterization. *Y. Jin[1], H. Zhang[2], C. Jin[1], Y.L. Kwong[2].* 1) Department of Clinical Genetics, University Hospital, Lund, Sweden; 2) Department of Medicine, the University of Hong Kong, Hong Kong.

Cell cultures of normal esophageal epithelium (NE) from four individuals were initiated and immortalized by the use of human telomerase reverse transcriptase (hTERT) alone or with the combination of human papilloma virus E6E7 in order to establish in vitro model to study the genetic alterations associated with cell immortalization and early neoplastic transformation of esophageal epithelium. All cell lineages transfected by a combination of hTERT and E6E7 could survive over crisis and eventually be immortalized, whereas only one of cell lineages transfected by hTERT alone could be immortalized. All these cell lineages were extensively characterized at different time-points before and after immortalization by sequential cytogenetic, molecular cytogenetic, and molecular genetic techniques. During early stage of crisis, there was a progressive increase of chromosomal aberrations in all cell lineages with the expression of E6E7, showing great genetic instability and cytogenetic divergence. Telomere association or dicentric chromosome was the major aberration types. In the later stage of crisis, all cells carried aberrations, but karyotypic picture tended to be more convergent. All cell lineages surviving over crisis had gain of chromosome 20 and/or increased copy number of 20q. By contrast, cell lineage immortalized by hTERT alone showed genetic stability throughout immortalization process. Molecular genetic investigations showed markedly deduction of expression level of RB and P53 after infection of E6E7 in all cell lineages. Cell lineage transfected only with hTERT had spontaneously downregulation of p16INK4a protein. In all cell lineages, the telomerase was consistently activated when cells approaching immortalization. Furthermore, Aurora A, which plays critical role in mitotic spindle checkpoint, was overexpressed in post-crisis cells immortalized by either method. Our findings indicate that different genetic mechanisms are involved in immortalization of NE cells.

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Oncogenic Transformation by SEI-1 is Associated with Chromosomal Instability. *X.Y. Guan, D. Tang, L. Hu, D. Xie, J.S. Sham.* Dept Clinical Oncology, Univ Hong Kong, Hong Kong, China.

Amplification of SEI-1, a cell cycle regulatory gene at 19q13.1 is commonly detected in ovarian cancer, suggesting a role in the pathogenesis of ovarian cancer. In the present study, the oncogenic potential of SEI-1 was demonstrated by anchorage-independent growth and tumor formation in nude mice with SEI-1-transfected NIH 3T3 mouse fibroblast cells. Silencing of SEI-1 gene expression by small interfering RNAs (siRNAs) in ovarian cancer cell line SKOV3 could inhibit cell growth as well as colony formation on soft agar. Chromosomal alterations including the formation of double minutes were observed in tumor cells derived from SEI-1-transformed NIH 3T3 cells. Micronuclei formation, which is an indicator of nuclear abnormality and genomic instability, was markedly increased in SEI-1-transfected cells. These data suggest that the oncogenic role of SEI-1 might be mediated at least in part via an effect on genomic instability. Furthermore, overexpression of SEI-1 was associated with higher tumor grades and late FIGO stages in ovarian carcinomas. These data strongly suggest that SEI-1 plays an important role in the development and progression of ovarian cancer.

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ADAM23, a Possible Tumor Suppressor Gene, is Frequently Silenced in Gastric Cancers by Homozygous Deletion or Aberrant Promoter Hypermethylation. *I.. Imoto[1,3], H. Takada[1,3], H. Tsuda[3,4], Y. Nakanishi[6], T. Ichikura[5], H. Mochizuki[5], F. Hosoda[3,7], S. Hirohashi[6], M. Ohki[7], J. Inazawa[1,2,3].* 1) Department of Molecular Cytogenetics, Medical Research Institute,Tokyo Medical and Dental University, Tokyo, Japan; 2) COE program for Frontier Research on Molecular Destruction and Reconstitution of Tooth and Bone, Tokyo Medical and Dental University, Tokyo, Japan; 3) Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology Corporation (JST), Saitama, Japan; 4) Department of Pathology II, National Defense Medical College, Tokorozawa, Japan; 5) Department of Surgery I, National Defense Medical College, Tokorozawa, Japan; 6) Pathology Division, National Cancer Center Research Institute, Tokyo, Japan; 7) Cancer Genomics Project, National Cancer Center Research Institute, Tokyo, Japan.

Array-based comparative genomic hybridization (CGH-array) has a powerful potential for high-throughput identification of genetic aberrations in cell genomes. We identified a homozygous loss of ADAM23 (2q33.3) in the course of a program to screen a panel of gastric cancer (GC) cell lines (1/32, 3.1%) for genomic copy-number aberrations using our custom-made CGH-array. Infrequent homozygous deletion of ADAM23 was also seen in primary gastric tumors (1/39, 2.6%). ADAM23 mRNA was expressed in normal stomach tissue, but not in the majority of GC cell lines without homozygous deletion of this gene. Expression of ADAM23 mRNA was restored to gene-silenced GC cells after treatment with 5-aza 2'f-deoxycytidine. The methylation status of the ADAM23 CpG island, which showed promoter activity, correlated inversely with its expression. Methylation of this CpG island was observed both in GC cell lines and in primary GC tissues; in primary tumors with a hypermethylated CpG island, expression of ADAM23 was lower than in adjacent noncancerous tissues. Moreover, restoration of ADAM23 in GC cells reduced their numbers in colony-formation assays. These results suggest that genetic or epigenetic silencing by hypermethylation of the ADAM23 CpG-rich promoter region leads to loss of ADAM23 function, which may be a factor in gastric carcinogenesis.

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Molecular Dynamics Simulations of BRCA1-BRCT Reveal Effects of Mutations on Protein-Protein Interaction Sites. *C. Gough[1], T. Imanishi[2], T. Gojobori[2,3].* 1) Japan Biological Information Research Center, Japan Biological Informatics Consortium, Tokyo, Japan; 2) Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan; 3) Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Mishima, Japan.

Mutations in the BRCT domain of BRCA1 (BRCA1-BRCT) are associated with increased risk of breast cancer. Cancer-associated mutations abolish BRCA1-BRCT's tumor suppressor function by disruption of interactions with other proteins such as the BACH1 helicase. Since concentrations of mutant BRCA1-BRCT *in vivo* are similar to that of the wild type, loss of function is not caused by diminished levels of folded protein, but likely is due to localized structural changes. To investigate this possibility, we performed molecular dynamics simulations on the three cancer-associated mutants A1708E, M1775R, and Y1853ter and on the wild type and the fully-functional variant containing the benign M1652I mutation, and analyzed the resulting backbone structures and fluctuation dynamics. The fluctuations were quantified with quasiharmonic analysis. The cancer-associated mutants exhibited backbone structure differences from the wild-type crystal structure, while the wild type and benign mutant remained close to the crystal structure. Structural differences were observed in BACH1-binding regions identified by published crystal structures and site-directed mutagenesis studies. In the cancer-associated mutants, the lowest-frequency, largest-amplitude quasiharmonic mode contained many widespread regions of large-amplitude fluctuations, in contrast to more localized amplitude peaks in the wild type and benign mutant. The lowest-frequency mode motions in the cancer-related mutants were qualitatively different from those in the wild type and M1652I mutant; the wild-type and M1652I motions consisted of concerted motions of structural elements, while those of the harmful mutants involved distortion of these elements. These mutation-induced structural and dynamic changes may be responsible for loss of BACH1 binding and tumor-suppressor activity.

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Mammographic Density and Breast Cancer Risk in BRCA1 and BRCA2 Mutation Carriers. *A. Antoniou[1], G. Mitchell[2], R. Warren[3], S. Peock[1], J. Brown[1], R. Davies[4], J. Mattison[1], M. Cook[1], I. Warsi[3], T. Cole[5], J. Paterson[5], S. Hodgson[5], L. Izatt[5], F. Douglas[5], G. Evans[5], D. Eccles[5], R. Eeles[6], D. Easton[1].* 1) CR-UK Genetic Epidemiology Unit, University of Cambridge, UK; 2) Peter Macallum Cancer Centre, Melbourne, Australia; 3) Dept of Radiology, Addenbrookes Hospital, UK; 4) Royal Devon and Exeter Hospital, UK; 5) EMBRACE study collaborators; 6) Institute of Cancer Research, Royal Marsden Hospital, UK.

High breast density as measured on mammograms is a strong risk factor for breast cancer in the general population, but its effect in carriers of germline BRCA1 and BRCA2 mutations is unclear. We obtained mammograms from 206 female carriers of BRCA1 or BRCA2 mutations, 96 of whom were subsequently diagnosed with breast cancer, and 136 relatives of carriers who were themselves non-carriers. We compared the mammographic densities of affected and unaffected carriers and of mutation carriers and non-carriers, using a computer assisted method of measurement and visual assessment by 2 observers. Analyses were adjusted for age, parity, body mass index, menopausal status and HRT use. There was no difference in the mean percent density between non-carriers and carriers (difference 2.9% for BRCA1 and 0.3% for BRCA2). Among carriers, increasing mammographic density was associated with an increased risk of breast cancer (p-trend=0.024). The odds ratio (OR) for breast cancer associated with a density of 50% was 2.29 (95% CI:1.23-4.26; p=0.009). The OR did not differ between BRCA1 and BRCA2 carriers or between pre- and post-menopausal carriers. Our results suggest that the distribution of breast density in BRCA1 and BRCA2 carriers is similar to that in the general population. High breast density in carriers is associated with an increased risk of breast cancer, with the relative risk being similar to that observed in the general population. This is consistent with the hypothesis that genetic and other risk factors that influence mammographic density and breast cancer risk in the general population also influence breast cancer risk to a similar relative extent in carriers. Use of mammographic density could improve individual risk prediction in carriers.

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The Clinical Breast Care Project: An important resource for studying breast cancer in African American women. *C.D. Shriver[1], V. Gutchell[1], L. Bronfman[1], L.A. Field[2], R.E. Ellsworth[2].* 1) Clinical Breast Care Project, Walter Reed Army Medical Center Washington , DC; 2) Clinical Breast Care Project Windber Research Institute Windber, PA.

The African American population is under-represented in tumor registries and clinical protocols. African American women (AAW) are affected with breast cancer at a younger age, tumor behavior is often more aggressive and survival times tend to be shorter. The Clinical Breast Care Project (CBCP) has developed a large tissue and blood repository from patients undergoing treatment for breast cancer, with previous history of breast cancer, counseled in the Risk Reduction Clinic, screened by routine mammography, or undergoing elective reductive mammoplasty. The success rate for recruitment of AAW is high; currently 24% of the 1,800 patients are African American, including 15% disease-free, 12% high-risk, 54% benign, 6% preinvasive and 13% invasive breast disease. More than 500 data fields regarding lifestyle choices, socioeconomic status, heath history and geography are collected from all participants, and all consenting individuals provide blood specimens for genomic and proteomic studies. Tissues are collected from all patients undergoing surgical procedures using protocols that preserve the macromolecules for downstream research applications. Factors such as education level, tobacco use, BMI, age at diagnosis, hormone receptor status, and BRCA1-like tumor characteristics differ significantly ($P<0.05$) between AAW and age- and stage-matched Caucasian women, reflecting differences seen in the general population. Thus, recruitment efforts in the CBCP have resulted in collection of well-annotated information and research-quality specimens from a large number of AAW. This resource is unique due to the number of quality samples, accompanying demographic information, equal-access to quality health care provided by the CBCP and will allow for the identification of biological and environmental factors associated with adverse clinical outcomes often seen in AAW with breast cancer.

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Evaluation of the worldwide-largest HNPCC cohort genotype-phenotype correlations, evidence for a new genetic entity. *E. Holinski-Feder[1,2], E. Mangold[2], C. Engel[2], H.K. Schackert[2], M. von Knebel-Doeberitz[2], T. Goecke[2], J. Willert[2], H. Vogelsang[2], M. Grabowski[1,2], C. Pagenstecher[2], S. Krueger[2], M. Kloor[2], G. Moeslein[2], M. Loeffler[2], C. Tympner[2], P. Propping[2].* 1) Institute of Human Genetics, Ludwig-Maximilians-University Munich, Muenchen, Germany; 2) HNPCC Consortium Germany www.hnpcc.de.

Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most frequent hereditary cancer syndromes defined by the fulfilment of the Amsterdam criteria. In 1999 a multi-centre study for the collection of HNPCC families aiming the further scientific workup was launched by the German cancer aid which resulted in the largest HNPCC cohort worldwide. So far, 2300 families meeting the Bethesda criteria, including 591 Amsterdam pedigrees have been documented. 470 pathogenic mutations in MSH2, MLH1 and to a minor extend in MSH6 have been found. Mutation analysis uncovered c.1489_1490insC in exon 13 of MLH1 and c.942+3A>T splicing defect in exon 5 in MSH2 covering app. 13% of all pathogenic mutations detected. Studies on genotype-phenotype correlation for 1381 tumors of 988 patients from 281 families within the MMR-deficient cohort revealed that MLH1 mutation carriers have a younger age of onset than MSH2 mutation carriers in regard of first cancer (41 vs. 44 y) and to first CRC cancer (42 vs. 46 y). The rate of CRC was higher in MLH1 versus MSH2 mutation carriers. The study cohort provides clinical and molecular evidence that the Amsterdam positive cohort includes two different genetic entities of hereditary CRC predisposition: 1)families with MMR-deficiency caused by mutations in DNA mismatch repair genes. 2)families with MMR-proficient tumors based on so far unknown genetic alterations. The main statistically significant clinical differences are the age of onset for all tumors observed (median 41 vs. 55 y, p 0,003), CRC (median 42 vs. 56 y, p 0,001), the localization of CRC tumor disease (p 0,006), the spectrum of associated tumors and disease progression. A different surveillance program for these families will be discussed. By analysis of chromosomal instability, the MMR-proficient cohort can be further subdivided.

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Untangling the association between the androgen receptor and prostate cancer risk. *A. Helgason[1], J. Gudmundsson[1], A. Sigurdsson[1], D.N. Magnusdottir[1], P. Sulem[1], M. Jakobsdottir[1], J.P. Kostic[1], B.A. Agnarsson[2], K.R. Benediktsdottir[2], S. Lindstrom[3], F. Wiklund[3], H. Gronberg[3], G.V. Einarsson[2], R.B. Barkardottir[2], J. Gulcher[1], U. Thorsteinsdottir[1], L. Amundadottir[1], K. Stefansson[1].* 1) deCODE Genetics, Reykjavik, Iceland; 2) University Hospital,Reykjavik, Iceland; 3) Umea University, Umea, Sweden.

Variation in the androgen receptor (AR) gene has been identified by a number of studies as a risk factor in the development of prostate cancer (PrCa). However, inconsistent results have cast some doubt on the role of the AR in PrCa -risk. We used the HapMap data to explore the pattern of haplotype diversity in and around the AR gene. Our results show that the AR gene is contained within a large LD block characterized by extremely divergent haplotype clusters, with great frequency differences between the HapMap populations. Four SNPs can be used to define the three major haplotype clusters observed in the region. Two of these haplotypes showed a marginally significant excess in Icelandic PrCa patients. By examining the evolutionary context of two previously unreported AR intron microsatellites in the HapMap samples, we noted a single haplotype, defined by one SNP and one microsatellite, that tagged both of the at-risk SNP haplotypes. When tested in the Icelandic case-control cohort this haplotype was seen in statistically significant excess in cases (RR= 1.57, p = 0.0017), with RR= 1.75 in high grade cancers (p = 0.0101) and RR= 1.83 in early-onset cases (p= 0.0042). We report findings from a replication analysis in a set of Swedish PrCa cases and controls. The at-risk haplotype tags one of the three basic haplotype clusters in the AR LD block, which is common in individuals of African ancestry, relatively rare in European populations and very rare in East Asian populations. Haplotypes belonging to this cluster tend to have shorter alleles for the two tri-nucleotide repeats in exon 1, than those from other clusters. Our results indicate that it is not length variation in the tri-nucleotide repeats, but rather older haplotype variants that underlie the association between AR and PrCa risk, at least in populations of European ancestry.

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Mutation frequencies for BRCA1 and BRCA2 in German breast cancer families indicate additional predisposing genes. *A. Meindl[1], M. Zaino[2], M. Brosig[2], R.K. Schmutzler[3], and German Consortium for Breast and Ovarian Cancer.*
1) Gynaecology and Obstetrics, Klinikum rechts der Isar, Munich, Germany; 2) Institute for Statistics and Epidemiology at the University, Leipzig, Germany; 3) Gynaecology and Obstetrics, Womens Hospital at the University, Cologne, Germany.

Purpose: In order to determine the mutation prevalence of the BRCA1 and BRCA2 gene for different famial risk constellations, we performed screening in 3081 affected females. Methods: Mutation screening was performed by either DHPLC, direct sequencing and application of the MLPA technique in affected females belonging to different risk groups. The latter technique was used to identify genomic deletions in the BRCA1 gene. Results: The highest mutation frequencies were detected in families presenting with breast and ovarian cancer (about 50%). Interestingly, a similar mutation rate was found in single females diagnosed for breast- and ovarian cancer. In families with three or more cases of breast cancer, two of them diagnosed before the age of 50, a mutation frequency of about 35% was observed. Even in families with 5 or more cases of breast cancer, including two premenopausal cases, the mutation frequency was only about 45%. High mutation rates for BRCA1/2 were also found in single females diagnosed before the age of 35 or in females diagnosed with bilateral breast cancer (approx. 20 or 30%, respectively). A low mutation prevalence (about 12.5%) was found for families with three or more cases of late onset breast cancer. Furthermore, even in families with five or more cases of breast cancer diagnosed after 50, the mutation prevalence is only about 15%. Conclusions: Based on our comprehensive screening results, we determined a cut off of 10% empirical mutation probability for offering genetic testing. Moreover, we demonstrated that families with multiple cases of late onset of breast cancer show a low prevalence of mutations in th BRCA genes. Finally, the existence of further predisposing genes is indicated by the lack of BRCA mutations in about 50% of families with multiple early- and/or late onset cases of breast cancer. Currently employed strategies to identify such genes will be discussed.

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Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. *J.L. Stanford[1], S.K. McDonnell[2], D.M. Friedrichsen[3], E.E. Carlson[2], S. Kolb[1], K. Deutsch[4], M. Janer[4], L. Hood[4], E.A. Ostrander[5], D.J. Schaid[2].* 1) Division of Public Health Sciences, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Mayo Clinic College of Medicine, Rochester, MN; 3) Divisions of Human Biology and Clinical Research, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 4) Institute for Systems Biology, Seattle, WA; 5) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

Prostate cancer is a heterogeneous disease, both genetically and phenotypically. Linkage studies attempting to map genes for hereditary prostate cancer (HPC) have proved challenging, and one potential problem contributing to this challenge is the variability in disease phenotypes. We collected clinical data on 784 affected men with prostate cancer from 248 HPC families for whom a genomic screen was performed. Disease characteristics (i.e., Gleason score, stage of disease, diagnostic PSA level, death from prostate cancer before age 65) were used to classify affected men into categories of clinically insignificant, moderate, or aggressive prostate cancer. These phenotypes were then incorporated into the linkage analyses using both a dominant and recessive model of inheritance. In addition, subset analyses considered age at diagnosis, number of affected men per family and other stratifications to try to increase genetic homogeneity. Several regions of interest (heterogeneity LOD score, HLOD > 1.0) were identified in families with > 2 affecteds with more aggressive prostate cancer. Suggestive linkage was observed at chromosome 22q11.1 (Dominant model HLOD = 2.18) and the result was stronger (Dominant HLOD = 2.75) in families with evidence of male-to-male disease transmission. A second region at 22q12.3-q13.1 was also highlighted (Recessive model HLOD = 1.90) in the aggressive subgroup, as was a region on chromosome 18. These analyses suggest that using clinically defined phenotypes may be a useful approach for simplifying the locus heterogeneity problems that confound the search for prostate cancer susceptibility genes.

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A genome wide linkage search for breast cancer susceptibility genes. *P. Smith[1], N. Rahman[2], M.R. Stratton[2], G.J. Mann[3], G.M. Pupo[3], P. Devilee[4], M. Southey[5], C. Szabo[5], D.E. Goldgar[5], D.F. Easton[1], BCLC, kConFab, CFRBCS.* 1) Public Health & Primary Care, University of Cambridge, Cambridge, UK; 2) Institute of Cancer Research, UK; 3) University of Sydney, Australia; 4) University of Leiden, Netherlands; 5) International Agency for Research on Cancer, Lyon, France.

Mutations in known breast cancer susceptibility genes account for a minority of the familial aggregation of the disease. To search for further breast cancer susceptibility genes, we performed a combined analysis of four genome-wide linkage screens, which included a total of 149 multiple case breast cancer families. All families included at least three cases of breast cancer diagnosed below age 60 years, at least one of whom had screened negative for BRCA1 and BRCA2 mutations. Evidence for linkage was assessed using parametric linkage analysis, assuming both a dominant and a recessive mode of inheritance, and using non-parametric methods. The highest LOD score obtained in any analysis was 1.80 on chromosome 4 (close to marker D4S392). Three further LOD scores over 1 (on chromosomes 2, 5 and 22) were identified in the parametric analyses and two (on chromosomes 2 and 14) in the non-parametric analyses. A maximum LOD score of 2.40 was found on chromosome 2p in families with four or more cases diagnosed below age 50. Nine LOD scores of >1.5 were obtained in individual families. These results suggest regions that may harbour novel breast cancer susceptibility genes, but indicate that no gene is likely to account for a large fraction of the familial aggregation of breast cancer.

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SF-1 Overexpression in Childhood Adrenocortical Tumors. *B.R. Haddad[1], M.A.D. Pianovski[2], L.R. Cavalli[1], B.C. Figueiredo[2], S.C.L. Santos[1,2], M. Doghman[3], R.C. Ribeiro[4], E. Michalkiewicz[5], G. Zambetti[4], E. Lalli[3].* 1) Georgetown Univ., Washington, DC; 2) Federal Univ. of Parana, Curitiba PR, Brazil; 3) Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne France; 4) St. Jude Children's Res. Hospital, Memphis TN; 5) Erasto Gaertner Hospital, Curitiba PR, Brazil.

We have recently shown that the SF-1 (steroidogenic factor 1) gene is amplified in pediatric adrenocortical tumors (ACT) (JCEM 90: 615-619, 2005). SF-1 encodes a transcriptional activator belonging to the nuclear hormone receptor superfamily and plays a pivotal role in the regulation of adrenal gland development and expression of steroidogenic enzymes. To assess whether SF-1 gene copy number increase translates into increased protein levels and to study the correlation between SF-1 expression and clinical parameters in ACT patients, SF-1 protein levels and gene copy number changes were measured in adrenocortical tumor specimens of 10 children, 6 girls and 4 boys, aged from 11 months to 11 years old. These children presented with only virilization (n=7) or virilization associated with Cushing syndrome (n=3). Histologically, 3 were adenomas and 7 carcinomas. All featured the R337H TP53 mutation, inherited from one of the parents, and loss of heterozygosity (LOH) at the TP53 locus in the tumors. We detected increased SF-1 gene copy number by FISH analysis in eight of the ten cases. Conversely, the SF-1 protein was overexpressed in all cases, compared to normal adrenal cortex; no significant correlation was found between SF-1 protein levels and gene copy number. Furthermore, no correlation existed with histological grade or with the clinical manifestation or evolution of the disease. These data allow us to propose the hypothesis that SF-1 protein overexpression may play an important role in driving ACT genesis and/or progression and that, in addition to gene amplification, epigenetic mechanisms may control SF-1 gene expression in pediatric ACT.

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Association of a histological type of gastric adenocarcinoma with an interleukin 1 beta promoter polymorphism: Interaction involving *Helicobacter pylori* CagA. *B. Schneider[1], L. Lopez-Carrillo[2], M. Camargo[1], P. Correa[1], R. Sierra[1], R. Henry[1], J. Chen[3], J. Zabaleta[1], M. Piazuelo[1], L. Sicinschi[1].* 1) Dept Pathology, LSU Health Sciences Ctr, New Orleans, LA; 2) Mexico National Institute of Public Health, Cuernavaca, Morelos, Mexico; 3) Mount Sinai School of Medicine, New York, NY.

Gastric adenocarcinomas are classified histologically into two major types: intestinal, in which the tumor cells form glands; and diffuse, in which tumor cells grow independently. Both types have as risk factors gastric infection with *Helicobacter pylori*. The intestinal type is preceded by a series of premalignant lesions: gastritis, glandular atrophy, complete intestinal metaplasia, incomplete intestinal metaplasia, and dysplasia. Diffuse gastric cancer has less well-defined premalignant lesions.

In European populations, SNPs in the interleukin 1 beta gene (*IL1B*) promoter are associated with gastric cancer risk. To examine the effect of *IL1B* polymorphisms, and any association with tumor type, we examined 183 cases of gastric adenocarcinoma (53% diffuse) and 377 hospital-based controls, all from Mexico. We genotyped *IL1B* (-31 and +3954), interleukin 1 receptor antagonist (VNTR) and interleukin 10 (*IL10*, -592). We evaluated serologically each person for the presence of *H.pylori* and CagA (an *H.pylori* virulence determinant). In intestinal tumors only, among patients with CagA positive *H.pylori* infections, we found a significant association of *IL1B* (-31) CC genotype (OR 3.19, 95%CI=1.05-9.68), compared to *IL1B*-31TT carriers. A significant interaction was found for *IL1B*-31C and CagA status in intestinal-type tumors (p=0.023). The *IL10*-592CC genotype was associated with elevated risk of intestinal-type gastric cancer (OR, 2.20, 95%CI=1.04-4.65) compared with the AA genotype. No associations were found for diffuse tumors. The risk of intestinal-type gastric cancer is apparently modified by *IL1B* or some gene in tight linkage with it; this process is amplified by the presence of CagA. In contrast, the risk of diffuse gastric cancer is unrelated to the state of the marker.

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Disruption of circadian gene expression in hepatocellular carcinoma. *J.G. Chang[1], K.T. Yeh[2], M.Y. Yang[3], T.C. Liu[3], S.F. Lin[3], W.L. Chan[1], S.J. Kuo[2].* 1) Dept Molec Med, China Medical Univ Hosp, Taichung, Taiwan; 2) Departments of Pathology and Surgery, Changhua Christian Hospital, Changhua, Taiwan; 3) Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background and Aims: Circadian rhythm plays an important role in the regulation of digestive system. The human circadian rhythm is controlled by at least 9 circadian genes. The aims of this study are to understand the difference of these 9 circadian gene expression between hepatocellular carcinoma (HCC) tissues and non-tumor tissue, and to explore the mechanism resulting in the difference. Methods: we analyzed the expression of 9 circadian genes in 46 hepatocellular carcinomas (HCC) and paired non-cancerous tissues by real-time quantitative RT-PCR (qRT-PCR) and immunohistochemical methods and explore the mechanism of down-regulation of 9 circadian genes by direct sequencing and methylation PCR analyses. Results: The results showed that the expression levels of PER1, PER2, PER3, CRY2 and TIM were decreased in HCCs. The down-expression of these genes was not caused by genetic mutation, but parts (34.8%) of them were caused by promoter methylation. The down-expression of these circadian genes has no relationship with the expression of the cell cycle-control genes. Conclusions: The down-regulation of circadian genes results in disturbance of circadian rhythm in HCCs, which may disruption the control of the central pacemaker and benefit the survival of cancer cells and promoting carcinogenesis. Differential expression of circadian genes between non-cancerous and cancerous cells may provide a molecular basis for chronotherapy of HCC.

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The Role of Inherited Variation in the Apoptosis Pathway in Colorectal Cancer Risk. *R.R. George[1], S. Brown[4], I.J. Adam[4], A.J. Shorthouse[4], T. Bishop[2], G. Smith[3], A. Cox[1].* 1) Genomic Medicine, University of Sheffield, Sheffield, Yorkshire, United Kingdom; 2) Cancer Research-UK Clinical Centre, St James' Hospital, Leeds, United Kingdom; 3) Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee, Scotland; 4) Department of Colorectal Surgery, Northern General Hospital, Sheffield, UK.

Colorectal cancer (CRC) is the third commonest cancer affecting Western Countries. Although there are inherited forms of the disease, the majority of cases are not familial. Disease in these cases is caused by a combination of weak effects at several genes and exposure to environmental risk factors.

Apoptosis is an essential defence against hyperproliferation and aberations of this process are hallmarks of cancer[1].

We have carried out a population-based case-control study of CRC to investigate single nucleotide polymorphisms in key apoptosis genes: caspase-8 (D302H, T21914C, A50121G, G50258A), caspase-10(L522I) and death receptor-4 (R209T). The study is based on a previously described set of 352 cases and 590 controls from the North of England and Scotland [2]. Blood DNA was genotyped by 5 nuclease PCR (Taqman). We found that there was a significant difference in the CASP8 T21914C genotype frequency between cases and controls (p=0.039). Carriage of the CASP8 C allele was found to be protective for CRC(odds ratio 0.54 - 95% confidence interval 0.33,0.89). We are in the process of genotyping further SNPs in the CASP8 gene to fine map this association. Furthermore, we are undertaking a replication study in a group of Sheffield-based cases and controls.

Indentifying which aspects of the apoptosis pathway are relevant in CRC susceptibility could suggest new targets for treatment.

[1]Evan et al(2001) Proliferation, cell cycle and apoptosis in cancer. Nature 411, 342-88

[2]Barret et al(2003)Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. Carcinogenesis 24, 275-82 .

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Germline Mutations in BRCA1 and BRCA2 from Korean Breast Cancer Patients by DHPLC. *D.G. Lee[1], B.Y. Kim[1], S.H. Han[2], K.R. Lee[1].* 1) Dept Human Genome, Seoul Medical Science Inst, Seoul, Korea; 2) Department of Internal Medicine, Ewha Womans University Medical Center, Seoul, Korea.

In Korea, the incidence of breast cancer has been increasing in recent years, such that it is now the most common female cancer. BRCA germline mutations cause a substantially increased lifetime risk of both breast and ovarian cancers. To investigate the presence of BRCA mutations in Korean breast cancer patients, we performed mutational analysis using DHPLC. From 2002 to 2005, we screened 370 breast cancer patients who were not selected on the basis of family histories and 167 individuals considered as not being at risk. We identified fourteen deleterious mutations: 10 frameshift mutations and 4 nonsense mutations. Among the 14 mutations, 3 novel BRCA1 (Q310X, 2167delA, 2478delG) and 3 novel BRCA2 (2487delT, 8662delGGA, R3384X) were identified in this study. We found that BRCA deleterious mutations were present in 19 of 370 patients (5.1 %). Three mutations (K467X, 3972delTGAG, R2494X in BRCA2) of the 14 deleterious mutations were detected in 7 of 19 patients, which were also identified in other studies for BRCA mutations in Korean population. Among the BRCA variants found in 370 patients, 7 BRCA1 variants (2201T, 2430C, 2731T, 3232G, 3667G, 4427C, 4956G) and 9 BRCA2 variants (203A, 1093C, 1342A, 1593G, 2457C, 3199G, 3624G, 4035C, 7470G) of high frequency were shown similar frequency in normal control group. Most of patients with deleterious mutations do not have a family history of breast cancer and observed only 4 patients with a family history of breast cancer. For immunohistochemistry, BRCA-associated tumors were shown to have more PR, p53, and less Her-2/neu positivity. Above findings were not in accordance with previous studies except Her-2/neu. In this study, we identified six novel BRCA mutations, and no recurrent founder mutations were observed, which are different from those of Ashkenazi Jew and other populations, but we found three mutations identified in other studies for Korean population. We suggest the fundamental data for large population-based studies to establish the frequency and significance of BRCA mutations in Korean breast cancer patients.

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Thymidylate synthase polymorphism (6bp-deletion) frequency in breast cancer from Mexican population. *M. Gallegos Jr[1], N.C. Olivares[1], G. Morgan[2], A.M. Puebla[1], V. Peralta[1], L. Arnaud[1], G.M. Zuniga[1].* 1) Dept Med Molec, Guadalajara, CIBO, IMSS, Jalisco, Mexico; 2) Unidad de Radio Diagnostico, Hospital de Especialidades, CMNO, IMSS.

Thymidylate synthase (TS; EC 2.1.1.45) is an enzyme essential for the provision of a nucleotide required for both DNA synthesis and repair, to participate in the proliferating cells and also is an important target for a variety of chemotherapeutic drugs, including 5-FU. Thus, TS plays a major role in cancer therapy and possibly in cancer prevention. Genetic polymorphisms in the TS gene may result in altered enzyme function. This could affect cancer susceptibility as well as treatment efficacy and the toxicity of antifolate cancer therapeutics. To date, of the frequency of genetic polymorphisms in TS identified as 6-bp deletion in the 39-UTR of the TS gene are unknown in Mexican population. The frequency of this polymorphism was confirmed in breast cancer patient (n=136) and a control group (n=100) from Mexican population by polymerase chain restriction amplification/RFLP analysis. The genotype frequency of the 6-bp deletion was found to be 30% in the patients group and 15% en the control group (OR 2.3 [CI95% 1.1-4.8] p=0.015), heterozygote of 45% and 30% respectively and the wildtype of 40% in both groups. The 6-bp deletion showed association in breast cancer patients from Mexican population with a risk of 2.3 times more than control groups.

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Most familial cancers appear to be heritable. *J. Lorenzo Bermejo[1], K. Hemminki[1,2].* 1) Division of Molecular Genetic Epidemiology, German Cancer Research Centre, Heidelberg, Germany; 2) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden.

Cancer is mainly an environmental disease, with a minor heritable etiology. The effects of common exposures shared by family members and heritable genes are reflected in the increased risks which have been observed for relatives of affected individuals. The present study investigated the contribution of shared environmental factors to the familial clustering of cancer. The acquired information was used to evaluate the feasibility of case-control studies on SNPs. We used the nationwide Swedish Family-Cancer Database and other published information. For example, the risk of lung cancer in the offspring of lung cancer patients was about twice higher than the risk in the general Swedish population. When published data on the heritability of smoking habits (0.5), the smoking prevalence (40%) and the odds ratio of lung cancer for smokers versus nonsmokers (20) were modeled, the relative risk of lung cancer attributable to smoking was 1.19. We show figures for the assessment of genetic parameters of most common cancer types. The results suggest that, in the absence of strong risk factors of cancer, most familial cancers are attributable to heritable genes. Unless established environmental risk factors are tested on proven candidate genes, the likelihood of a successful application of SNPs in gene-environment studies is small.

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Transfection of in vitro-transcribed EGFP-mRNA into SK-N-AS and 293 cells shows major difference in the sensitivity to a nuclear compared to a cytoplasmic EGFP-variant. *K. Ejeskär[1,2], S. Fransson[1], F. Zaibak[2], P. Ioannou[2].* 1) Clinical genetics, Gothenburg university, Gothenburg, Sweden; 2) Murdoch Childrens Research Institute, Melbourne University,Parkville, VIC, Australia.

Good transfection efficiency and low toxicity are two important factors for reliable results in in vitro gene studies. Here we report a method for efficient transfection of in vitro transcribed mRNA into two different types of adherent cells, the neuroblastoma cell line SK-N-AS, and the transformed kidney cell line 293. By using newly trypsinized adherent cells in suspension and LipofectamineTM 2000 we can detect a transfection efficiency of 80-90% in both cell lines, and a cell viability of 90% in SK-N-AS and 60% in 293, 24 hours after transfection when using cytoplasmic enhanced green fluorescent protein (EGFP)-mRNA. We have also evaluated the different effects of the generally used EGFP that mainly localizes to the cytoplasm and nuclear EGFP, where the nuclear EGFP are significantly more toxic to the cells than the cytoplasmic EGFP. In order to develop a good null experiment for functional tests of nuclear proteins we have constructed a short non-functional mRNA including a nuclear localisation signal, and evaluated at what concentrations mRNA encoding nuclear proteins can be added without a general toxicity only depending on the fact that the protein are localised to the nucleus. For both SK-N-AS and 293 cells, a concentration of up to 100 ng mRNA in 105 cells, encoding a non harmful nuclear protein, does not affect the cells. We have developed an easy method for efficient delivery of in vitro transcribed mRNA into the adherent cell lines SK-N-AS and 293. This method is useful for functional screening of genes encoding both nuclear and cytoplasmic proteins.

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Role of biomarkers in the development of breast cancer. *D.O. McDaniel[1], S. Bigler[2], W.H. Barber[1], A. Lewis[1].* 1) Dept Surgery, Univ Mississippi Medical Ctr, Jackson, MS 39216; 2) Dept Pathology.

This study is to identify genetic variants of the Cytochrome P450 (CYP) 3A4 gene that might be used as diagnostic biomarkers in studies of breast cancer susceptibility. Biochemical pathways involved in hormone metabolism have been implicated in the etiology of breast cancer and the CYP 3A enzymes are involved in such metabolisms. A transition of AG at position -290 of the CYP 3A4 gene has an effect on the level of gene transcription and has been associated with several disease conditions. We sought to determine the frequency distribution of this variant and its effect on gene expression in breast cancer. Patients undergoing lumpectomy, needle localization, simple mastectomy and modified radical mastectomy were recruited in this study. Clinical conditions including menopausal status, tumor stage, receptor status, previous breast cancer and family history were evaluated for genotype and phenotype association. Genotypes were detected by a PCR-base approach using SNP analysis of human CYP 3A4 gene. Expression levels of mRNA transcripts were determined by a semiquantitative RT-PCR. The homozygous AA allele was present with higher frequency (71.0%) in Caucasian (CAU) group as compared with African American (Af-Am) (29%) patients and controls (15%). Homozygous GG alleles were absence in CAU patients and the frequency was increased 4.8-fold in cancer patients as compared with controls ($p<0.05$). A vs. G genotypes were inversely associated with tumor staging in Af-Am patients. Patients with stage 0 tumors 50% carry the AA alleles as compared with 33% in stage II, whereas patients with stage II and III, 67 to 100% carry AG or GG alleles. The expression levels of CYP 3A4 transcript was higher in PBMCs of patients with benign tumors as compared to malignant tumors ($p<0.03$). In summary, the CYP 3A4 genotype demonstrated a remarkable interindividual variation between Af-Am and CAU patient populations, furthermore, Af-Am patients with homozygous GG genotype were at higher risk of developing cancer as compared with controls. Thus, the CYP 3A4 genotype analysis may predict likelihood of developing breast carcinoma, and might allow earlier detection of cancer.

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Splicing defects in hereditary nonpolyposis colorectal cancer. *I. Tournier[1], A. Martins[1], C. Martin[2], J. Auclair[3], Q. Wang[3], M.-P. Buisine[4], S. Olschwang[5], T. Frebourg[1,2], M. Tosi[1].* 1) Inserm U614, IFRMP, Faculty of Medicine, Rouen, France; 2) Department of Medical Genetics, Rouen University Hospital; 3) Centre dOncologie Génétique, Centre Léon Bérard, Lyon, France; 4) Laboratoire de Biochimie et de Biologie Moléculaire, CHU Lille, France; 5) Institut Paoli-Calmettes, INSERM UMR 599, Marseille, France.

Hereditary nonpolyposis colorectal cancer (HNPCC)is the major form of hereditary colorectal cancer and results from constitutional mutations in mismatch repair genes, especially *MLH1* and *MSH2*. One of the current limitations of the molecular diagnosis of HNPCC results from the numerous missense or translationally silent mutations in these genes and from the difficulty of establishing simple functional assays for the corresponding proteins. Considering that some of these variants could be pathogenic by affecting splicing, our present goal is to optimise the detection of all splicing defects of *MLH1* and *MSH2*, including the characterisation of defects possibly affecting ESE or ESS elements. We have listed in France more than 120 distinct missense or translationally silent mutations of *MLH1* or *MSH2*. In order to evaluate the potential effect of these mutations on splicing, we have undertaken a parallel screening of these mutations using focused RT-PCR analysis of RNA obtained from patient peripheral blood and a functional assay of exon inclusion or exclusion. The latter is based on an expression construct, driven by a CMV promoter, into which exons 2 and 3 of the *SERPIN/G1* gene, separated by their natural intron, were inserted. In this assay, wild type and mutant exons are PCR amplified from patient DNA together with about 150 bp of flanking sequences, cloned into the intron of the expression construct and transiently transfected into HeLa cells. The effects of mutations on splicing are evaluated by RT-PCR analysis of the resulting mRNA and direct sequencing of the RT-PCR products. Several mutations located near the 3 exon boundaries, previously classified as missense, were found to be in fact splicing defects. Complete analysis of this cohort will provide an evaluation of the impact of mutations of these genes affecting ESE or ESS elements.

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A new microarray-based diagnostic tool for resequencing the entire BRCA1 and BRCA2 genes. *M. Bonin Sr, O. Altug-Teber, F. Stutzmann, S. Poths, O. Riess.* Medical Genetics Department, Institute for Human Genetics, Tuebingen, Germany.

It has been 10 years since the BRCA1 gene was first identified. During this decade, genetic testing for breast cancer susceptibility has been incorporated into the practice of oncology. In this process, the identification of families at the highest hereditary risk for cancer has served as a model to test strategies for prevention or early detection of breast malignancies. Here we report the first microarray-based resequencing analysis of the complete BRCA1 and BRCA2 genes. For this purpose, we used the oligonucleotide-microarray technology and designed a CustomSeq-Array for the coding region of both genes. Twenty-five unrelated patients and control persons were analysed. All exons of each sample were amplified by PCR using specific primers, pooled, labelled, fragmented, and hybridised to the BreastCancer-CustomSeq array. In addition all samples were confirmed by conventional sequencing procedure. All analysed BRCA mutations could be detected by the new diagnostic system. The BreastCancer-Array provides base calls at more than 99.5 accuracy which is comparable to capillary sequencing. Replicate experiments demonstrated a reproducibility of more than 99.95%. We conclude that array-based sequencing technology has the capability to efficiently and cost-effectively generate large-scale resequencing data of genes. The technology is in particular applicable to large genes with numerous different mutations, like BRCA, but is also utilized for highly heterogenous diseases. Furthermore CustomSeq arrays deliver a complete sequence within 48 hours which opens a revolutionary new era of sequence-based diagnostics.

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Genome-wide SNP association studies for interstitial lung disease in gefitinib (Iressa)-treated patients with non-small cell lung cancer. *T. Mushiroda[1], Y. Ohnishi[2], S. Saito[1], A. Takahashi[1], N. Kamatani[1,3], Y. Nakamura[1,2].* 1) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 3) Division of Genomic Medicine, Department of Applied Biomedical Engineering and Science and Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Drug-associated interstitial lung disease (ILD) is severe and sometimes fatal among non-small cell lung cancer (NSCLC) patients treated with gefitinib (Iressa). We conducted exploratory case-control association studies using gene-based 52,608 single nucleotide polymorphisms (SNPs) with 102 NSCLC patients receiving gefitinib (28 patients with ILD, 74 without) to identify genes susceptible to ILD. We observed associations with p-value of < 0.01 at 306 SNP loci calculated by allele frequency model ($p < 0.0001$, 3 SNPs; $p < 0.001$, 30 SNPs), at 129 SNP loci by dominant inheritance model ($p < 0.0001$, 1 SNP; $p < 0.001$, 5 SNPs), and at 293 SNP loci by recessive inheritance model ($p < 0.0001$, 1 SNP; $p < 0.001$, 28 SNPs). The predictive validation using a novel block sequential method is ongoing based on a nested case-control study in Japan run by AstraZeneca, and any positive results will be evaluated for clinical utility to guide gefitinib therapy.

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Mutations in *FANCD2* are not prevalent in hereditary breast cancer families. *T. Scholl[1], K. Eliason[1], A. Carlson[2], R. Kalb[3], K. Neveling[3], H. Hoehn[3], H. Hanenberg[4], S.D. Batish[2], A. Parslow[1], H. King[1], B.C. Hendrickson[1], T. Judkins[1], M. Norton[1], D. Schindler[3], A.D. Auerbach[2].* 1) Myriad Genetic Laboratories, Salt Lake City, UT; 2) Laboratory of Human Genetics and Hematology, The Rockefeller University, NY; 3) Department of Human Genetics, University of Wuerzburg, Germany; 4) Department of Pediatric Oncology, Hematology and Immunology, University of Dusseldorf, Germany.

Fanconi anemia (FA) exhibits extensive genetic heterogeneity. Seven of the FA proteins (FANCA/B/C/E/F/G/L) form a nuclear FA core complex that activates the monoubiquitination of FANCD2, targeting FANCD2 into nuclear foci containing BRCA1, BRCA2/FANCD1 and RAD51. These functional associations between the *BRCA* and FA genes suggest a role for the FA pathway in the repair of DNA strand breaks and prompts the hypothesis that additional FA genes could also be responsible for hereditary breast cancer. Familial cancer histories in pedigrees from the International Fanconi Anemia Registry (IFAR) were examined for incidence of breast, ovarian, and pancreatic cancers. Six families with mutations in the *FANCD2* gene that appeared to contain especially high incidences of these cancers were selected for further study. Within these families, mutations in *FANCD2* did not appear to segregate in subjects affected with cancer in this ongoing study. In addition, direct DNA sequencing was employed in families with strong histories of breast/ovarian cancer to screen for mutations in the 9 exons of *FANCD2* which contained mutations in the IFAR pedigrees. This specimen set comprised 420 fully anonymous specimens derived from probands affected with breast/ovarian cancer prior to age 50 and also reported at least two relatives affected with breast/ovarian cancer that were previously shown to be negative for mutations in *BRCA1* and *BRCA2* as part of clinical genetic testing. No obvious pathogenic mutations in these regions of *FANCD2* were detected, although some missense variants of uncertain clinical significance were observed. These results support the conclusion that *FANCD2* mutations probably do not play a significant role in hereditary breast cancer.

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Results of a *BRCA1/BRCA2* Mutation Screening Panel in 119 Women of African ancestry. *L. Gayol[1], M.E. Ahearn[1], T. Donenberg[2], B. Morel[1], J.F. Arena[2,3], L. Baumbach[1].* 1) Dept Pediatrics, Miller School of Medicine, University of Miami, Miami, FL; 2) FOBCC, Miller School of Medicine, University of Miami, Miami, FL; 3) National Cancer Institute, Washington, D.C.

Recent evidence from our laboratory as well as others, suggest that certain *BRCA1/BRCA2* mutations and variants appear to be either at increased frequency or specific to women of African ancestry (AA) at-risk for breast cancer. These observations have led to development of a screening panel for selected *BRCA1* and *BRCA2* mutations/variants in AA women. This panel is designed to detect 37 *BRCA1* mutations and unclassified variants, and 22 *BRCA2* mutations and unclassified variants, either previously detected in our laboratory, or found in a thorough review of all published English literature. The screening strategy is based on a combination of DNA sequencing and SSCP. Currently, the mutation-screening panel is comprised of five DNA sequencing reactions for *BRCA1*, and four DNA sequencing reactions for *BRCA2*, which together detect 86% of the mutations and variants, the remainder being detected by SSCP. In the 119 patients analyzed, only two deleterious (and recurrent) *BRCA1* mutations were detected: the founder mutation *BRCA1* 943ins10 and a splicing mutation *BRCA1* IVS13+1 G>A, both at a 3.3% frequency. In *BRCA2* gene, we detected just one rare deleterious mutation: 6828 del TT. A number of other recurrent missense mutations, unclassified variants and polymorphisms were also detected in both genes; three in *BRCA1* and six in *BRCA2*. In addition, five rare missense mutations were detected in one patient each. The frequency of these variants is being investigated. Based on these data, we are refining the mutation-screening panel for application to a population-based study.

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Mutation screening of BRCA1 and BRCA2 in the Netherlands and Belgium: An overview of 9 years screening and identification of 1700 mutation positive families. *F.B.L. Hogervorst[1,2], H. Gille[2], A. van der Hout[2], G. Vink[2], M. Vreeswijk[2], P. Devilee[2], A. van den Wijngaard[2], R. Blok[2], D. Bodmer[2], M. Ligtenberg[2], H. Bruggenwirth[2], A. van den Ouweland[2], R. van der Luijt[2], K. Claes[2], G. Michils[2], E. Teugels[2], S. Wilcocx[2].* 1) Family Cancer Clinic, Dept Pathology, Netherlands Cancer Inst, Amsterdam, Netherlands; 2) The Dutch/Belgian working group on BRCA mutation screen of the Clinical Genetic centers.

In 1995 the DNA-diagnostic laboratories of the Dutch and Belgian Clinical Genetic Centres started DNA testing for BRCA1 and BRCA2. At present, BRCA1 and BRCA2 are completely screened either by a combination of PTT and DGGE/DHPLC or entirely by DGGE/DHPLC/Sequencing. In addition BRCA1 specific testing is performed by Multiplex Ligation-dependent Probe Amplification (MLPA). Up to March 2005, more than 10.000 families have been tested identifying 1700 mutation positive families: 1190 BRCA1 and 510 BRCA2 families. For BRCA1, 171 distinct pathogenic mutations have been identified. The TOP 10 mutations did account for almost 55% of the families. For BRCA2 173 distinct mutations were identified of which the majority (127) is found in only one or two families. The TOP 10 mutations account for 42% of the families. For both genes we identified regional and national founders. Recently, BRCA1 mutation screening has been improved by a novel method, called MLPA. This test allows rapid screening for rearrangements in the BRCA1 gene in a high throughput format. In addition to the genomic deletions of exons 13 or 22, more than 15 different deletions and duplications have been found. With the completion of the mutation scanning for both genes many so-called unclassified variants (UV's) have been reported. These are minor changes in the genes for which the relation with the genetic predisposition for breast/ovarian cancer still has to be established. For BRCA1, 148 different UVs have been identified in 241 families. For BRCA2 this is number is much larger: 247 different UVs in 481 families. As more than 80% of all different UVs have been reported only once or twice this clearly is a drawback in the elucidation of the pathogenic status of such variants.

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The prevalence of *MSH6* mutations in North American patients receiving clinical genetic testing for HNPCC.

B.C. Hendrickson, A.P. Parslow, B. Wardell, J.T. McCulloch, K. Eliason, T. Judkins, A.E. Deffenbaugh, J. Tazelaar, W.W. Noll, B.E. Ward, S. Bhatnagar, T. Scholl. Myriad Genetic Laboratories, Inc., Salt Lake City, UT.

The protein encoded by *MSH6* is involved in DNA mismatch repair and heritable *MSH6* mutations convey risk for colorectal cancer. It has been reported that mutations in *MSH6* may convey less risk than those in *MLH1* or *MSH2*, two other DNA repair genes associated with HNPCC. Mutations in *MSH6* may also be preferentially associated with other cancers, particularly endometrial cancer. It is difficult to estimate the importance of *MSH6* mutations in HNPCC since the prevalence that has been reported varies greatly between studies. This variation probably reflects biases due to the particular effects of *MSH6* mutations mentioned above combined with the criteria employed to select patients for particular research studies. Our objective is to determine the overall prevalence of *MSH6* mutations in North American patients undergoing clinical genetic testing for HNPCC.

A set of 381 specimens previously submitted for clinical genetic testing by direct DNA sequencing and Southern blot analysis of *MLH1* and *MSH2* were selected for this study. The initial testing had identified 64 deleterious mutations and 38 genetic variants of uncertain clinical significance in *MLH1* and *MSH2*. Of the remaining specimens, 279 had sufficient material for further analysis and were made fully anonymous for *MSH6* mutation screening. *MSH6* analysis included direct DNA sequencing for the entire coding region and consensus splice sequences adjacent to the exons. A total of six protein truncating mutations were detected, which would indicate an overall *MSH6* mutation prevalence of 2.2%, or 11.4% of all mutations detected in these samples. In addition, seven missense variants of uncertain clinical significance were detected.

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Protein truncation testing to identify *BRCA1* and *BRCA2* variants of unknown significance that are splicing mutations. *J.K. Booker[1], L.R. Susswein[2], C. Skrzynia[3], N. Adams[4], J.P. Evans[2,3].* 1) Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; 2) Genetics, University of North Carolina, Chapel Hill, NC; 3) Hematology Oncology, University of North Carolina, Chapel Hill, NC; 4) Moses Cone Regional Medical Center, Greensboro, NC.

Molecular genetic testing is increasingly sequence based, with the advantage of detecting rare mutations in addition to those that are more common and generally targeted by simpler mutation detection methods. While sequencing has advantages in the number of mutations that can be detected, it has the disadvantage of identifying all sequence variants, including benign polymorphisms and variants of unknown significance (VUS). VUS are, by definition, uninterpretable with regard to their contribution to the cause or risk of disease and present a complicated and unsatisfying result to patients, counselors and physicians. When a VUS is identified, clues to disease association can be found by looking at segregation of the variant with disease, and evolutionary conservation and degree of chemical change when an amino acid is altered, but this information is not definitive. Here we used the protein truncation test to evaluate the splicing consequences of one *BRCA1* missense mutation (5191 C>T, T1691I), one *BRCA2* silent mutation (459 T>G, T77T), and two *BRCA2* missense mutations (457 A>G, T77A and 9599 A>T, N3124I). Three of the four VUS were shown to be associated with a truncated protein. Sequencing of the cDNA used to generate the truncated protein demonstrated that the truncated protein resulted from abnormal mRNA splicing. Abnormal splicing was presumed to result from interruption of exonic splicing enhancers. Prediction of the exact splice product was not possible based on the exonic location of the variant, illustrating the need to look beyond the immediate flanking exons, when a splicing mutation is suspected. Characterizing a silent or missense mutation identified through sequencing as truncating with the protein truncation test can alter the interpretation from a VUS to a probable deleterious mutation and is thus an important adjunct to mutational analysis.

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Contribution of BRCA1 and BRCA2 germ-line mutations to the incidence of breast and ovarian cancer in young Cypriot women. *A. Hadjisavvas[1], M. Loizidou[1], Y. Marcou[2], D. Papamichael[2], M. Televantos[3], G. Kalakoutis[3], K. Kyriacou[1].* 1) Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus; 2) Oncology center Bank of Cyprus, Nicosia , Cyprus; 3) Makarios III Hospital, Nicosia, Cyprus.

In Cyprus, the contribution of the BRCA mutations to the population incidence of early-onset breast cancer was unknown. We carried out a mutational analysis of the BRCA1 and BRCA2 genes in 30 Cypriot women diagnosed with breast cancer before the age of 40 and 15 women diagnosed with ovarian cancer before the age of 60 irrespective of their family history. Genetic analysis was performed by PCR and DNA sequencing. We identified 5 pathogenic mutations in the BRCA genes, in 7 unrelated probands: 2 in BRCA1 (K614X and 5424delG) and 3 in BRCA2 (3758del4, 6310insA and 8984delG). Two out of the 5 mutations are novel (BRCA2 3758del4, 6310insA) and two were previously identified in Cypriot high risk families (BRCA1 5424delG and BRCA2 8984delG). The BRCA2 8984delG mutation was detected in three non-related breast cancer patients. Additionally, 2 new missense mutations were identified (BRCA1 H628Y and BRCA2 I2068L). In total, 5 out of 30 early-onset breast cancer patients carried a pathogenic BRCA mutation (20%) which is high in comparison to the expected 5-10%. This higher than expected frequency, may be a result of the presence of the already identified Cypriot founder mutation in the BRCA2 gene. Regarding the ovarian cancer patients, 2 out of 15 (13%) carried pathogenic BRCA mutation which is within the expected range. These results confirm that in the Cypriot population the BRCA2, rather than BRCA1 gene appears to play a more significant role in the breast cancer phenotype. This study has indicated that Cypriot women with early-onset breast cancer, irrespective of their family history, are a good predictor of being a BRCA mutation carrier.

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Predicting *BRCA1* and *BRCA2* genotype by expression profiling after induced DNA damage:a novel approach for functional mutation detection? *Z. Kote-Jarai[1], L. Matthew[2], A. Osorio[1,3], I. Giddings[2], I. Locke[1,4], S. Shanley[1,4,5], R. Williams[6], C. Campbell[7], R. Eeles[1,4].* 1) Translational Cancer Genetics, The Institute of Cancer Research, Sutton, Surrey, UK; 2) Molecular Carcinogenesis, The Institute of Cancer Research, Sutton, Surrey, UK; 3) Department of Human Genetics, Spanish National Cancer Centre, Madrid, Spain; 4) Royal Marsden NHS Foundation Trust, London, UK; 5) Westmead Hospital, Sydney, Australia; 6) Pediatrics Oncology, The Institute of Cancer Research, Sutton, Surrey, UK; 7) Computational Intelligence Unit, University of Bristol, Bristol, UK.

Germline mutations in *BRCA1* and *BRCA2* predispose women to an increased risk of breast and ovarian cancer. Both genes have important roles in DNA damage repair and in gene expression regulation. We have studied the differential gene expression profiles after radiation-induced DNA damage in normal cells from *BRCA1/2* mutation carriers using high density microarray technology. Short-term primary fibroblast cultures were established from skin biopsies from healthy women: 10 *BRCA1* and 10 *BRCA2* mutation carriers, and 10 non-carrier control individuals. The cells were subjected to high dose ionizing irradiation (15 Gy) to induce DNA damage. We have used 15K spotted cDNA microarrays manufactured by the Cancer Research UK Microarray Facilities. For data analysis Significance Analysis of Microarrays (SAM), hierarchical clustering and the Support Vector Machine classifier (SVM) was applied. SAM and SVM identified the best genotype predictor features for *BRCA1* and *BRCA2* heterozygous genotype. The supervised class prediction model, SVM and leave-one-out (LOO) cross validation, performed with high accuracy (85-95%), *BRCA1* and *BRCA2* carriers could be clearly distinguished from non-carriers and from each other after radiation induced DNA damage. Without irradiation however no significant differences were found, providing further evidence that these genes have important roles in DNA damage responses. We propose that gene expression profiling could provide a novel functional mutation detection technique that could have important implications in the identification functionally important alterations in these genes.

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Frequency of the mutation IVS14+IGA in the dihydropyrimidine dehydrogenase gene in cancer patients from Mexican population. *V. Peralta[1], MR. Flores[2], G. Morgan[3], L. Arnaud[1], E. Vargas[1], M. Gallegos[1].* 1) Div. Medicina Molecular, CIBO, IMSS, Guadalajara,, Jalisco, Mexico; 2) Departamento de Patologias, Hospital de Especialidades, CMNO, IMSS; 3) Unidad de Radio Diagnostico,Hospital de Especialidades, CMNO, IMSS.

The dihydropyrimidine dehydrogenase (DPD), is the first enzyme that takes part in the degradation of 5-fluorouracil (5FU), related to toxic effects in cancer patients that receive this antineoplasic as part of the chimeoterapeutic scheme. One of the most frequent mutations of the dihydropyrimidine dehydrogenase gene (DPYD), is the called IVS14+IGA, that results in a deletion of exon 14 by a change of G instead A in the splicing site GT '5 and as consequence a non functional protein. Objective: To determine the frequency of the mutation IVS14+IGA of the gene DPYD from Mexican cancer patients. Material and methods: We analized 102 DNA samples of cancer patients, from the Oncology Division of HE,CMNO, IMSS and 117 DNA samples as control group from general population of the West of Mexico. Results: The genotypic frequency was in Hardy Weinberg's equilibrium in the groups of study. The wild type genotype was observed in 88.2%; in the patients group, in comparison to 100%; of control group. The heterocygote genotype was observed in 10.8%, and homocygote for the mutation in 1%; in the patients group. When to compared the study groups were statistically significant difference (p=0.0001) with Odds ratio for 15.86 (95%CI 2.15-327). Conclusions: The mutation IVS14+IGA showed association in Mexican cancer group with a OR of 15.86 times major with regard to the normal genotype.

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Increasing detection efficiency of microsatellite instabilities in colon carcinoma by applying a label-free method.

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Microsatellite instability (MSI) is caused by a failure of the DNA mismatch repair system and occurs frequently in various types of cancer. Since MSI, associated with ca. 10 to 15 % of colorectal, gastric or endometrial carcinoma, impact clinical prognosis, MSI analysis is an important tool of molecular pathology. This study aimed to develop a simple and efficient procedure of MSI detection. 40 cases with no (27), low (l) or high (h) MSI (13), pre-identified by conventional fluorochrome-associated PAGE technology, were selected out of a panel of 150 patients with colon carcinoma. Microdissected non-tumor (N) and tumor (T) tissue areas of one or two 4 m-sections were de-paraffinized and DNA was extracted. Primer sequences recognizing the five microsatellite loci BAT25, BAT26, D5S346, D17S250, D2S123, were selected according to the recommendation of the 1997 National Cancer Institute-sponsored conference on MSI. Primer sets were applied in label-free duplex or single PCR assays for DNA amplification and amplicons were analysed by microfluidics based on-chip electrophoresis. In all 40 cases, chip linked microcapillary electrophoresis of the amplicons, arisen from tumor and non-tumor DNA, resulted in highly resolved, distinct patterns of each of the microsatellite loci. Label-free detection of MSI could be demonstrated by microsatellite loci-associated deviations in the electropherogram profiles of tumor and non-tumor material, and confirmed the prediagnosis of the MSI cases by conventional technology. Here, we present a simple and robust approach for MSI detection, which allows a label-free microsatellite analysis of uncharacterized microdissected tissue areas within 30 minutes.

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Genetic basis of meningioma formation and progression is significantly different between men and women. *F.P. Nunes[1], I. Ahronowitz[1], A.O. Stemmer-Rachamimov[2], M. MacCollin[1].* 1) Molecular Neurofibromatosis Laboratory, Massachusetts General Hospital, Charlestown, MA; 2) Molecular Neuro-Oncology Laboratory, Massachusetts General Hospital, Charlestown, MA.

Meningiomas are common neoplasms of the brain and spinal cord with an annual incidence of 7.8 per 100,000 individuals. Interestingly, meningiomas are much more prevalent in women, with a female to male ratio of 2.3:1. We sought to determine if genetic differences might be associated with this disparity by studying 131 sporadic meningioma patients (91 women and 40 men) randomly selected from the Massachusetts General Hospital Neuro-Oncology Tumor Bank. Full record review included clinical data, radiology reports, and pathology reports. Loss of heterozygosity (LOH) at the neurofibromatosis 2 (*NF2*) locus was determined using polymorphic microsatellite markers on chromosome 22q. In addition, LOH of 1p, 10q, and 14q, previously shown to be associated with meningioma recurrence, was determined in a subset of 45 patients. Average age at surgery was similar between men (55.3 years) and women (57.5 years) in this cohort. Meningiomas of higher grades were more common in men (WHO grade II and III, 40%) than in women (32%, $p< 0.10$). *NF2* LOH was seen in 56% of cases, but was more often present in tumors from men (70% of cases) than in those from women (50% of cases, x^2 Test = 4.27, $p<0.05$). Interestingly, men with tumors lost at *NF2* had an average age at surgery of 52.9 years, versus 61.7 years in women ($p<0.05$). LOH of 1p, 10q, and 14q were seen in 7 (16%), 2 (4%), and 8 (18%) tumors, respectively, and were associated with *NF2* loss in 10 out of 13 cases. Losses of 1p and 14q were more common in tumors from men (21% and 20%) than women (13% and 17%), but this finding did not reach statistical significance. Our results show clear differences in the molecular biology of sporadic meningiomas from men and women. Our current work is focused on further understanding these differences with the hypothesis that this will explain the increased risk of women for meningioma development.

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HLA DRB1 gene and cervical squamous cell carcinoma in Chinese. *T.Y. Chang[1], Y.C. Yang[1,2,4], Y.J. Lee[1,3,5], T.H. Su[2,4], C.W. Dang[1], H.F. Liu[1], C.C. Chu[1], M. Lin[1].* 1) Department of Medical Research; 2) Gynecology and Obstetrics; 3) Pediatrics, Mackay Memorial Hospital, Taiwan; 4) Mackay Medicine, Nursing and Management College; 5) College of Medicine, Taipei Medical University, Taiwan.

Cervical cancer is the second most common cancer in women in developing countries and the second most common cancer worldwide. Polymorphisms in human leukocyte antigen (HLA) genes have been implicated in the risk for cervical cancer. Association of HLA alleles, particularly class II, with cervical cancer in different ethnic populations has been documented. The association, however, is scarcely reported in Asian populations. We examined HLA-DRB1 gene frequency in 123 women with cervical squamous cell carcinoma (CSCC) and 298 healthy control subjects. All patients and control subjects were Chinese living in Taiwan. Genotyping of HLA-DRB1 gene was carried out by sequence-based typing. An increase in the frequency of the DRB1*1502 and a decrease in the frequency of the DRB1*12 were observed in patients, however, the differences were not statistically significant as compared to controls (P=0.06). Relevant studies on the association between HLA-DRB1 and CSCC were searched and retrieved from MEDLINE database and analyzed. The results from literature search demonstrated that DRB1*1001, DRB1*1101, and DRB1*1501 were associated with increased risks and DRB1*0301, DRB1*1301/02 were associated with decreased risks. Some literatures also showed that the frequency of DRB1*12 had decreased tendency in patients but without statistical significance. Given the literature results we could sensibly infer that DRB1*1502 might be an allele making some Chinese women more susceptible to CSCC. In addition, the trend of decreased frequency of DRB1*12 found in patients was in accordance with the published data. In conclusion, this is the first study investigating the association between HLA-DRB1 gene and CSCC in Chinese. Although the association between HLA and CSCC was unable to be confirmed in our population, the possibility of a very weak association still remains. Further studies by extensive genotyping of other polymorphic sites of the HLA genes in a larger dataset are necessary.

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MYH Y165C and G382D mutations in hepatocellular carcinoma and cholangiocarcinoma patients. *L.M. Baudhuin[1], L.R. Roberts[2], F.T.B. Enders[3], R.L. Swanson[1], T.A. Mettler[2], I. Aderca[2], L.M. Stadheim[2], W.E. Highsmith[1].*
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Purpose: Production of reactive oxygen species (ROS) during chronic inflammation has been implicated in the progression of liver diseases and carcinogenesis. Subjects with inflammatory liver disease and one nonfunctional allele of the base excision repair gene, MYH, may be more susceptible to progression to cancer due to MYH haploinsufficiency in repairing oxidative damage caused by ROS. Furthermore, a common area of loss of heterozygosity (LOH) in hepatocellular carcinoma (HCC) is at 1p34-36, which is near the chromosomal location of MYH (1p32.1-34.1). Here, we investigated the association of two common germline MYH mutations in patients with HCC and cholangiocarcinoma. **Methods:** DNA from patients with HCC (n=48) or cholangiocarcinoma (n=84) compared to non-cancerous controls (n=308) were genotyped by restriction fragment polymorphism analysis for the common Y165C and G382D mutations in MYH. Fisher's Exact Test was used to compare patient population data to control data. **Results:** There was no significant difference in MYH mutation carrier status between patients with HCC (1/48), cholangiocarcinoma (3/84), and non-cancerous controls (4/308). One patient with cholangiocarcinoma and a monoallelic MYH mutation also had inflammatory bowel disease. **Conclusions:** Patients with HCC or cholangiocarcinoma do not have an increased incidence of monoallelic germline MYH mutations predisposing them to disease. However, somatic and/or other germline MYH mutations involved in disease pathogenesis of HCC or cholangiocarcinoma cannot be ruled out. Further investigations are necessary to determine whether MYH mutations are involved in progression of inflammatory bowel disease.

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Examination of the effect of MC1R gene variants on melanoma risk in CDKN2A mutation carriers across continents : A Melanoma Genetics Consortium (GENOMEL) study. *F. Demenais, for the Melanoma Genetics Consortium.* INSERM EMI-0006, Evry, France.

Melanocortin-1 receptor (MC1R) gene plays a key role in human pigmentation. MC1R polymorphisms have been shown to be associated with pigmentation phenotypes and with melanoma independently of skin type. Penetrance of CDKN2A, a gene predisposing to familial melanoma, was also found to be influenced by MC1R variants. We examined the effect of MC1R variants on melanoma risk in CDKN2A mutation carriers using data from 14 groups from Australia, Europe and North-America (the United States and Canada) that are part of The Melanoma Genetics Consortium. Logistic regression analysis was applied to the total sample of 768 CDKN2A mutation carriers (452 affected and 316 unaffected subjects) who were genotyped for MC1R and to subgroups of subjects according to geographical location (125 Australians, 450 Europeans, 193 North-Americans). Overall, the absolute number of MC1R variants carried, three common specific variants (R151C, R160W, V60L) as well as pooled RHC variants increased significantly melanoma risk : p-value being always less than 0.001, except for V60L and R160W for which p was equal to 0.02. A dosage effect of MC1R variants (2 variants conferring a higher risk than only one) was consistently observed across all three continents. When examining the effect of specific variants by continent, melanoma risk was always increased but significance was only reached in Australia and Europe for R151C (p0.01), in North-America for R160W (p=0.01) and in Australia for V60L (p=0.04). These effects of MC1R polymorphisms will be further evaluated by taking into account pigmentation characteristics and sun-related covariates.

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***LHFPL3*, a Candidate Gene for Uterine Leiomyoma.** *T.S. Ptacek[1], C.Y. Song[1], C.L. Walker[2], S.M. Sell[1].* 1) Department of Nutrition Sciences, UAB, Birmingham, AL; 2) Department of Carcinogenesis, UTMDACC, Austin, TX.

Uterine leiomyoma (UL), a benign smooth muscle tumor of the uterus, is common among women of reproductive age. Although often asymptomatic, UL can cause serious health problems. Deletions on chromosome band 7q22 are common in UL. Recently, we reported a minimal 7q22 deletion interval defined by closely spaced microsatellite markers (Sell *et al.*, 2005). One marker, D7S2446, was deleted, and microsatellite instability (MSI) was observed at this maker. Based on the May 2004 human genome assembly, this interval contains two genes: the origin recognition complex 5-like (*ORC5L*) and lipoma *HMGIC* fusion partner-like 3 (*LHFPL3*) genes. The *ORC5L* gene has been previously studied as a candidate gene for UL in tumor samples with larger deletions (Quintana *et al.*, 1998). The *LHFPL3* gene is a member of a new family of *LHFP-like* genes. Another member, the *LHFP* gene, is a fusion partner with the *HMGIC* gene in lipoma (Petit *et al.*, 1999). In this study we used bioinformatics following a tiered approach (Ptacek and Sell, 2005) and expression analyses to examine the *LHFPL3* gene as a candidate gene for UL. The D7S2446 marker was positioned within the first intron of the *LHFPL3* gene. The human gene showed sequence identity with the mouse and rat (exon 1: >95%, exons 2 and 3: >80%) and the genomic organization was conserved across species. Analyses of the protein sequence identified four transmembrane helix domains, including a signature sequence for the peripheral myelin protein 22 (PMP22) Claudin family. Members of the PMP22 Claudin protein family participate in extracellular matrix formation, proliferation and differentiation. *LHFPL3* expression, assessed using RT-PCR and the Agilent 2100 Bioanalyzer, was either significantly increased or decreased in human UL compared to matched, normal myometrium (n=8 total; $P < 0.05$). Similar results were obtained using the Eker rat (UL, n = 8; normal myometrium, n = 4; $P < 0.05$). We conclude that the *LHFPL3* gene is a candidate gene for UL. Future studies will assess the possible molecular pathogenic roles of *LHFPL3* MSI and the observed distinct patterns of gene expression.

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Polygenic models, association studies and bilateral breast cancer. *J. Peto[1,2], N. Johnson[3], O. Fletcher[1], C. Lombardelli[3], I. dos Santos Silva[1], A. Ashworth[3].* 1) EPH, LSHTM, London, United Kingdom; 2) Cancer-Research UK Epidemiology & Genetics Unit, ICR, Surrey, United Kingdom; 3) The Breakthrough Breast Cancer Research Centre, London, United Kingdom.

Bilateral breast cancers have important advantages for detecting susceptibility alleles in association studies. They provide considerably more power than unselected cases, and unlike cases with several affected relatives, they can be ascertained systematically through cancer registries. The proportions of bilaterals and unaffected controls who carry a susceptibility allele can thus be used to calculate unbiased risk estimates for the general population. Under a polygenic model in which many low-penetrance alleles interact to produce a wide spectrum of individual susceptibility, bilaterals and other familial breast cancer patients are at particularly high risk because on average they carry more susceptibility alleles than unselected cases. An allele that interacts synergistically with other (as yet unknown) alleles should therefore confer a high absolute risk among bilateral patients and their relatives. Findings based on the first 469 bilateral cases: Under a polygenic model, each allele should be more common and the absolute risk in carriers should be higher in bilateral than in unselected cases, and also in their relatives. The first degree relatives of our bilateral cases suffered substantially larger excesses of prostate cancer (SIR=2.4 95%CI 1.7-3.4) and breast cancer (SIR=3.5 95%CI 3.0-4.1 in women, 15.1 95%CI 4.9-35.4 in men) than relatives of unselected cases. CHEK2*1100delC, which is carried by about 1% of Caucasians, doubles the risk of breast cancer in women unselected for family history. Relatives of bilateral cases who were CHEK2*1100delC carriers had greatly increased risks for both breast cancer (SIR=12.1 95%CI 5.2-23.9) and prostate cancer (SIR=9.9 95%CI 1.2-35.7), suggesting that CHEK2*1100delC interacts with unidentified low penetrance polygenes. CHEK2 carrier status may be a clinically important determinant of absolute risk in women with bilateral disease or a strong family history of breast cancer.

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Molecular Classification Of 39 Cancer Subtypes With A Gene Panel: Implications For Diagnosis Of Carcinoma Of Unknown Primary. *R.D. Patel, J. Murage, R.M. Desai, R.C. Salunga, S.S. Chu, X. Wang, R. Raja, X-J. Ma, M.G. Erlander.* Life Sciences, Arcturus Bioscience, Inc., Mountain View, CA.

Identifying the origin of a carcinoma of unknown primary (CUP) enables a physician to more effectively treat a CUP patient. Using current immunohistochemistry methods, the origin of only ~25% of CUPs is usually identified. Gene expression-based classifications have recently been shown to be highly successful in predicting the origin of cancer types. These previous molecular classifications of cancer types have been completed via whole-genome microarray-based gene expression profiling using frozen tissue. However, neither the use of whole-genome microarrays nor the assaying of frozen tissue is always practical in the real-world. Therefore, we have developed a gene panel amenable to PCR-based quantification utilizing formalin-fixed paraffin-embedded (FFPE) tissue for the prediction of the primary origin of metastatic cancers. A total of 497 cancers (training set: 75% primary, 25% metastatic) representing 39 cancer types were expression-profiled by a whole-genome array (Agilent). Following this, a bioinformatic strategy was used to identify a set of <96 genes for the prediction of the origin of 39 cancer types. A set of 87 genes (plus 5 reference genes) from the microarray data was identified as having the ability to predict the origin of 39 cancer types. To validate this set in-silico, 146 FFPE samples representing these 39 cancer types were used as a test set using real-time PCR, in which 83% sensitivity and 99% specificity was achieved. Subsequently, a PCR-based training and test database consisting of 339 frozen and 61 FFPE tumor samples was generated for 31 cancer types (a subset of the 39) and 85% sensitivity and 99% specificity was achieved. We have demonstrated that the primary origin of a large number of different cancer types can be predicted with a relatively small number of genes (87 genes/39 cancer types = ~2 genes/cancer type) using routine clinical samples. This approach should lead to an assay that is amenable for a clinical laboratory that utilizes 96-well PCR-based assays.

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Strong evidence for linkage with prostate cancer at chromosome 17q in the University of Michigan Prostate Cancer Genetics Project pedigrees: Eighty-nine new pedigrees provide additional evidence for a chromosome 17q susceptibility locus. *E.M. Lange[1], C. Robbins[2], L.A. Ho[1], K.A. White[3], Y. Wang[1], W.B. Isaacs[4], K.A. Cooney[3].* 1) University of North Carolina, Chapel Hill, NC; 2) TGen, Phoenix, AZ; 3) University of Michigan, Ann Arbor, MI; 4) John Hopkins Medical Institutions, Baltimore, MD.

Previously, Lange et al. (Prostate, 2003) described a genome-wide linkage scan (GWS) for 175 prostate cancer pedigrees from the University of Michigan Prostate Cancer Genetics Project (UM PCGP). The maximum LOD score (2.36) using non-parametric linkage methods (the pairs allele-sharing statistic and Kong and Coxs exponential model) occurred at chromosome 17q between markers D17S798 and D17S1868 in near proximity to the breast cancer susceptibility gene BRCA1. Herein, we extended our analyses on chromosome 17q to include 12 additional microsatellite markers and 89 additional prostate cancer pedigrees with three or more confirmed prostate cancer cases. The additional markers marginally increased the LOD to 2.41 in the original GWS families, with the maximum LOD score occurring near the new marker D17S1788 in the same location as the peak in the original GWS. The 89 new pedigrees gave a maximum LOD score of 0.77 (p=0.03), which also occurred at marker D17S1788. Combining the original GWS pedigrees with the 89 new pedigrees resulted in a maximum LOD = 3.07 at D17S1788. There was evidence for linkage in both our Caucasian pedigrees (n = 242; LOD = 2.35 at D17S1788) and African American pedigrees (n = 19; LOD = 1.01 at D17S1868 and LOD = 0.45 at D17S1788). Finally, consistent with previous results, the maximum evidence for linkage occurred in the subset of pedigrees with four or more confirmed prostate cancer cases, LOD = 4.11 near D17S1868, approximately 7.5 cM distal to D17S1788. Two recent linkage studies that have combined pedigrees from different groups including our original GWS families (Gillanders et. al, 2004, JNCI; Xu et. al, 2005, AJHG) have implicated chromosome 17q as a candidate interval for a prostate cancer susceptibility locus. Neither of these studies included the corroborating data from these 89 new pedigrees.

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Further evidence of linkage on chr5 in a large Tasmanian prostate cancer family. *J. McKay[1,2], L. Fitzgerald[1], A. Banks[1], K. Hazelwood[1], J. Brohde[3], G. Hannan[3], T. Dwyer[1,4], J. Dickinson[1].* 1) Menzies Ctr, Biochem, Univ Tasmania, Hobart, Tasmania, Australia; 2) International Agency for Research on Cancer, Lyon, France; 3) PHealth, CSIRO, Nth Ryde, Sydney, Australia; 4) Murdoch Research Institute, Melbourne, Victoria, Australia.

The Tasmanian familial prostate cancer study's purpose is to use large extended pedigrees from the Australian island state of Tasmania to attempt to understand the genetics of familial prostate cancer. Recently, we have used the HMA10K high density SNPchip to perform a genome wide scan in a 6-generation pedigree in which 18 cases of prostate cancer could be traced back to a common founding pair. Through this genome scan we identified evidence for linkage at the centromere of chromosome 5, an area noted by other prostate cancer studies. Using further genealogical research, we have identified a second 5-generation pedigree from the same geographical region of Southern Tasmania, in which 11 cases are able to be traced back to a common founding pair. Interestingly, one patient is present in both pedigrees via marriage. Founder mutations have been noted in Tasmania, and given the limited population of this area and presence of two large pedigrees, an undiscovered genealogical link between these families seemed plausible. Genotyping of both SNP and microsatellite markers showed some evidence consistent with linkage at chr 5 in this second large family. However, we were unable to find evidence of a common founding haplotype among these two pedigrees.

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Evidence for Balancing Selection within an FHIT Intronic Region Implicated in Prostate Cancer. *Y. Ding, G.P. Larson, G. Rivas, L. Geller, C. Lundberg, C. Ouyang, T.G. Krontiris.* Dept Molecular Medicine, Beckman Res Inst, City of Hope, Duarte, CA.

Previously, we identified a locus for prostate cancer susceptibility at D3S1234 within FHIT (maximum LOD = 3.17, LODPAL) using a candidate gene-based linkage approach on 228 brother pairs (200 families) affected with prostate cancer. Subsequent association tests in Americans of European descent on 16 SNPs spanning approximately 400 kb surrounding D3S1234 revealed significant evidence of association for a single SNP (Pearson¡¦s ^{2} = 8.54, df = 1, p = 0.0035) within intron 5 of FHIT. Genotyping 40 tagging SNPs within a 30 kb region surrounding this SNP further delineated association of prostate cancer risk to a 10 Kb region. Population studies (13 Americans of European descent and 16 Yorubans) revealed strong signatures of balancing selection within the European population, but not within the African population. A sliding window analysis of resequencing data from individuals of European descent revealed a 13 Kb region of peaks and plateaus of Pi > 0.004 and Tajima¡¦s D > 2.0 (max. Pi = 0.0074, max. Tajima¡¦s D = 3.06, p < 0.001 under a standard neutral model). The elevated Pi and Tajima¡¦s D extends across three LD blocks, suggesting the possibility of multiple sites under selection. Decay of these D statistic elevations elsewhere suggests that population structure and past demographic events do not account for our result. Within the LD block associated with prostate cancer, the haplotype enriched in the control group is the most common haplotype in European descent (40%) compared to only 10% in the Yoruban population. In contrast, the putative risk haplotype is 28% in Americans of European descent and occurs as the most common haplotype (33%) within the Yoruban population. Our study, which suggests an important selectable function within intron 5, also represents an additional corroborative approach for gene-disease associations.

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Assaying for Loss of Heterozygosity in Tumor Samples using Relative Fluorescent Quantitation on Capillary Electrophoresis Systems. *S. Karudapuram[1], L. Joe[1], A. Wheaton[1], T. McElroy[1], Y. Lou[1], A. Rico[2], C. Stemmer[3], A. Schneider[3], M-P. Gaub[3], M. Legrain[3], P. Oudet[3].* 1) Applied Biosystems, Foster City, CA; 2) Applied Biosystems, France; 3) Service de Biochimie et de Biologie Moléculaire, Hôpital Hautepierre, Strasbourg, France.

Several capillary electrophoresis based fragment analysis applications require comparison of peak heights across samples as a relative quantitation method. Screening for Loss of Heterozygosity (LOH), Microsatellite Instability (MSI) and detection of chromosomal deletions and duplications are typical examples of such applications. Optimized chemistries, a robust and reliable electrophoresis platform as well as accurate analysis software are all essential to the success of these assays. In this study we use a LOH assay to demonstrate relative quantitation using capillary electrophoresis. Microsatellite markers were run on the Applied Biosystems 3130*xl* Genetic Analyzer and peak heights were compared across paired samples from tumor and healthy tissues. Using GeneMapper v3.7 Software samples were identified as LOH candidates based on a threshold enabling rapid identification for downstream review. Our results highlight the 3130 Series Systems in conjunction with GeneMapper Software as an optimal solution for relative fluorescent quantitation assays.

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Detection of ultra rare somatic mutations in p53 by Bi-PAP-A. *J. Shi, Q. Liu, S. Sommer.* Department of Molecular Genetics and Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA 91010-3000.

Detecting rare sequence variants in the presence of excess amounts of normal genomic DNA is central to i) early cancer detection; ii) cancer chemotherapy; iii) assessment of environmental mutagen exposure, and iv) prenatal diagnosis of paternally derived mutations within fetal cells in the maternal circulation. Bi-PAP-A (Bi-directional Pyrophosphorolysis Activated Polymerization Allele-specific Amplification) is a method that uses two opposing 3¢-terminal blocked oligonucleotides (P*s) with one nucleotide overlap at their 3¢ termini. The selectivity of Bi-PAP-A derives from the serial coupling of pyrophosphorolysis and DNA polymerization. Eighteen Bi-PAP-A assays were developed for the p53 gene. The sensitivity and specificity of each assay were tested using mutated and wild type DNA. Bi-PAP-A typically has a sensitivity of 1-2 molecules and selectivity greater than 10^7; in some cases, selectivity greater than 10^9 can be achieved. However, in the case of G:CA:T transitions and G:CT:A transversions, the selectivity is limited to about 10^4~10^5. Low selectivity is likely due to the deamination of cytosine during the amplification process and the presence of 8-oxo-dG in the genomic DNA, respectively. Efforts are under way to increase the selectivity of G:CA:T and G:CT:A, and to determine if Bi-PAP-A can be used to assay deamination of C and 8-oxo-dG damage in DNA. Five assays with high selectivity were used to detect rare somatic mutations in blood, enabling the detection of minimal residual disease at levels far below the sensitivity of conventional methods. The mutation frequency ranged from 10^{-7} to less than 10^{-8}. We conclude that Bi-PAP-A is a rapid and general method for detecting rare mutations although its selectivity may sometimes be limited by DNA damage.

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Genetic and epigenetic alterations at chromosomal region 11p in sporadic Wilms tumors. *H. Soejima[1], Y. Satoh[2], Y. Kaneko[3], T. Mukai[1].* 1) Division of Molecular Biology and Genetics, Department of Biomolecular Sciences, Saga University, Saga, Japan; 2) Department of Urology, Saga University, Saga, Japan; 3) Research Institute for Clinical Oncology, Saitama Cancer Center, Saitama, Japan.

Chromosomal regions 11p13 and 11p15.5 are known to be loci for Wilms tumors. 11p13 is called the WT1 locus and 11p15.5, the WT2 locus. The *Wilms tumor 1* gene (*WT1*), which is mutated in a minority of Wilms tumors, is mapped to 11p13 (WT1 locus). It is known that the *WT1* mutation is significantly associated with the -*catenin* (3p21) mutation. 11p15.5 (WT2 locus) is a well-known imprinted region that is divided into two domains, *IGF2*/*H19* and *KIP2*/*LIT1*. Loss of imprinting (LOI) of *IGF2*, reduced expression of *KIP2*, and aberrant methylation of imprinting centers (ICs) and of gene promoter regions have been reported in Wilms tumors. However, it has not been clear whether or how the genetic and epigenetic alterations of these four loci *WT1*, -*catenin*, *IGF2*/*H19*, and *KIP2*/*LIT1* are associated. In this study, we performed analyses for loss of heterozygosity (LOH), gene mutation, gene expression, imprinting status, and DNA methylation on a total of 37 sporadic Wilms tumors. We found 10 tumors with only genetic alterations, which were 11p LOH, *WT1* mutation, and -*catenin* mutation, and 18 with only epigenetic alterations, which were LOI of *IGF2*, reduced expression of *KIP2* and *WT1*, and aberrant methylation of ICs and of *WT1* promoter. Five tumors showed simultaneously the both genetic and epigenetic alterations. Thirty-three tumors (89%) showed at least one of the alterations of the four loci, and 19 tumors (51%) had alterations of multiple loci. However, there was no tumor showing only the -*catenin* mutation at 3p21. The results indicate the genetic and epigenetic alterations of 11p are involved in a majority of sporadic Wilms tumors, and suggest that these alterations play an important role in Wilms tumorigenesis.

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The role of mitochondrial complex II in hereditary neoplasia. *B. Battersby[1], S. Leary[1], T. Wai[1], G.H. Guercin[1], J. Green[2], E. Shoubridge[1].* 1) Human Genetics, MNI, McGill University, Montreal, Quebec, Canada; 2) Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

The mitochondrial respiratory chain is composed of five multimeric complexes required for aerobic ATP production. Complex II consists of four nuclear-encoded subunits: two membrane-bound subunits and two soluble subunits in the matrix of mitochondria. The complex functions in both the citric acid cycle, by oxidizing succinate to fumarate, and in the electron transport chain by transferring electrons from FADH2 to ubiquinone. Germline mutations in SDHB, SDHC, and SDHD have been identified in patients with hereditary head and neck paraganglioma and/or pheochromocytomas, suggesting that these proteins function as tumor suppressors. In the tumors, a somatic deletion of the wild-type allele results in a loss of complex II function, and activation of the hypoxia response pathway by stabilization of the transcription factor, HIF1-alpha. We have investigated the genetic basis for hereditary head and neck paraganglioma in a large Newfoundland pedigree and identified a novel mutation in SDHB that segregates with the disease. In tumor tissue, there is a loss of heterozygosity and a dramatic increase in the expression of HIF1-alpha target genes. Blue-native gel electrophoresis demonstrates that the mutant allele of SDHB prevents assembly of the mature holoenzyme, thereby eliminating complex II function in mitochondria. Mutations in the fourth subunit of complex II, SDHA, are associated with an autosomal recessive, early-onset, neurodegenerative disorder, called Leigh syndrome. It is currently unknown why the loss of complex II function in mitochondria can produce two very different clinical presentations. To investigate the mechanism behind these two pathologies, we used shRNA directed against the different complex II subunits to generate stable knockdowns that impair assembly of the complex. This genetic model system should allow us to identify the molecular mechanism of activation of the hypoxia response pathway and establish why only three of the four complex II subunits function as tumour suppressors.

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Mitochondrial DNA G10398A polymorphism and risk of invasive breast cancer in African-American women.

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Mitochondria generate oxygen-derived free radicals that damage mitochondrial DNA (mtDNA) as well as nuclear DNA and in turn promote carcinogenesis. The mtDNA G10398A polymorphism alters the structure of Complex I in the mitochondrial electron transport chain, an important site of free radical production. This polymorphism is associated with several neurodegenerative disorders. We hypothesized that the 10398A allele is also associated with breast cancer susceptibility. African mitochondria harbor the 10398A allele less frequently than Caucasian mitochondria, which predominantly carry this allele. Mitochondrial genotypes at this locus were therefore determined in two separate populations of African-American women with invasive breast cancer and in controls. A preliminary study at Vanderbilt University (48 cases, 54 controls) uncovered an association between the 10398A allele and invasive breast cancer in African-American women, OR=2.90 (95% CI 0.61-18.3; P=0.11). We subsequently validated this finding in a large, population-based, case-control study of breast cancer, the Carolina Breast Cancer Study at the University of North Carolina (654 cases, 605 controls). African-American women in this study with the 10398A allele had a significantly increased risk of invasive breast cancer, OR=1.60 (95% CI 1.10-2.31; P=0.013). The 10398A allele remained an independent risk factor after adjustment for other well-accepted breast cancer risk factors. No association was detectable in white women (879 cases, 760 controls), OR=1.03 (95% CI 0.81-1.31; P=0.81). This study provides novel epidemiologic evidence that the mtDNA 10398A allele influences breast cancer susceptibility in African-American women. MtDNA polymorphisms may be underappreciated factors in breast carcinogenesis.

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A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. *Y. Bai, J. Park, P. Hu, R. Xiang, D. Holstein, J. Lechleiter, S. Naylor.* Cellular & Structural Biol, Univ Texas Health & Sci Ctr, San Antonio, TX.

Mitochondrial alteration has been long proposed to play a major role in tumorigenesis. Recently, mitochondrial DNA (mtDNA) mutations have been found in various cancer cells. In this study, we have examined the contribution of mtDNA mutations to tumorigenesis with human cell lines carrying a frameshift mtDNA mutation on NADH dehydrogenase (respiratory complex I) subunit 5 (ND5) gene which was also identified in a colorectal cancer cell line. The anchorage dependence phenotype and tumor-forming ability of cells carrying wildtutype and mutant mtDNA were tested by growth assay in soft agar and subcutaneous implantation of the cells in nude mice. One cell line carrying the heteroplasmic ND5 mtDNA mutation showed significantly enhanced tumor growth, while cells with homoplasmic form of the same mutation inhibited tumor formation. We further provided evidence to suggest that these results might be mediated by alteration in reactive oxygen species (ROS) generation and apoptosis. Based on the data obtained, we propose that mtDNA mutations play an important role in tumorigenesis. Our working model for the role of mtDNA mutations in cancer development is as follows. In the initial stage, cancer cells are very mutagenic either because of a carcinogenic insult or the compromised repair mechanism and mtDNA is more likely to be mutated at this stage. Because of replicative advantage of the mutant mtDNA molecules, mtDNA mutations get enriched to certain level of heteroplasmy which would enhance tumor progression due to the elevated ROS generation which in turn activates the oncogenic pathways. In late stages of cancer, the cells are progressively adapted to a glycolytic metabolism because of the hypoxic environment. This may lead to the selection of cells in which the mutations makes mitochondrial function-independent, and therefore cells with homoplasmic mtDNA mutation may become predominant in tumors. However, when a homoplasmic status of some mtDNA mutations is reached, the host cells could be converted into non-tumorigenic cells because of the loss ATP production.

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Identification of a tumor-associated germline mutation in the mitochondrial DNA that reduces cytochrome c oxidase activity. *M.E. Gallardo[1], R. Moreno-LosHuertos[2], M. Casqueiro[1], J. Silva[3], F. Bonilla[3], J.A. Enriquez[2], S. Rodriguez de Cordoba[1].* 1) Department of Inmunology, Centro de Investigaciones Biológicas, CSIC, Spain; 2) Department of Biochemistry and Molecular Biology, Universidad de Zaragoza, Spain; 3) Department of Medical Oncology, Hospital Universitario Puerta de Hierro, Madrid, Spain.

To identify genetic variants in the human mitochondrial DNA potentially associated to tumors we have fully sequenced the mtDNA of thirteen randomly selected cell lines derived from a variety of human cancers. Among several mtDNA mutations, we found two that are functionally relevant, 12240delC and 6267G>A. 12240delC alters the mitochondrial tRNASer(AGY) and 6267G>A causes the Ala122Thr substitution in the Cytochrome c Oxidase subunit I (CO I). Biochemical analysis of the original cell lines and the transmitochondrial cybrids generated by transferring mtDNAs to a common nuclear background, demonstrate that COX activity, respiration and growth in galactose are impaired by the 6267G>A mutation. 6267G>A was found twice in the cancer cell lines. Interestingly, the 6267G>A mutation was also found in one out of 15 breast cancer samples analyzed in this report and it has been described previously in one out of 15 pancreatic cancer cell lines and in one out of 260 prostate cancer samples, always associated to different mtDNA haplogroups. In contrast, the 6267G>A mutation was encountered only once in a compilation of 2,064 full mtDNA sequences from the literature and was not detected in 200 additional non-cancer mtDNA sequences from our laboratory. These findings indicate that the CO I missense mutation 6267G>A (Ala122Thr), impairs COX activity and is a recurrent mutation specifically associated with cancer. Based on the analysis of matched tumor/normal DNA pairs from cancer patients we propose that 6267G>A is a mtDNA germline mutation predisposing to cancer.

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MtDNA sequence heterogeneity in single cells from leukemia patients. *Y.G. Yao[1], Y. Ogasawara[1], S. Kajigaya[1], J.J. Molldrem[2], R.P. Falcão[3], M.C. Pintão[3], J.P. McCoy[1], K. Keyvanafar[1], E.G. Rizzatti[3], N.S. Young[1].* 1) Hematology Branch, NHLBI, NIH, Bethesda, MD; 2) M.D. Anderson Cancer Center, Houston, TX; 3) University of Sao Paulo at Ribeirao Preto Medical School, Ribeirao Preto, Brazilã.

A high frequency of mtDNA somatic mutation has been observed in many tumors as well as in ageing tissues. Specific characteristics of malignant growths, especially generation of increased reactive oxygen species, may contribute to mitochondrial genetic alternations, leading to an increase in mtDNA heterogeneity among different cells. Conversely, rapid expansion of a clonal malignant cell population would act to either dilute mutations or to fix by genetic drift specific mtDNA changes, thus leading to lower overall heterogeneity. In this study, we analyzed the mtDNA control region sequence heterogeneity in single normal cells and individual blasts from leukemia patients, to address the mutation process in tumor cells and further to determine whether mtDNA be useful as a marker for monitoring disease progression and the presence of minimal residual leukemia. Among 1304 cells analyzed, we found a higher variance of the frequency of the single cells harboring mtDNA heterogeneity from the 13 patients (8.5% - 65.0%) compared to that of the 6 normal controls (36.6% - 44.1%). The 8 AML patients did not show a uniform pattern of heterogeneity in single blasts. One relapsed AML patient presented a complex shift of two different haplotypes in single cells, which might be the result of chemotherapy. Most mtDNA heterogeneity in single cells was caused by the C-stretch length mutations in the second hypervariable segment. Three patients showed high frequencies of heterogeneity at sites 189, 260, and insertion of the AC repeat at region 515-524, respectively, which could be considered as a marker for disease progression. Aging-dependent mutation accumulation observed in normal tissues was abrogated in leukemic blasts due to the blast expansion and crisis. Our results suggest that the somatic mutation processes in leukemia is complex, leading to deviations from normal level of heterogeneity due to either intrinsic aspects of leukemia pathophysiology and/or chemotherapy effects.

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Major regions of mitotic recombination of heterozygous mice fibroblasts located on chromosome 8. *I.V. Tereshchenko, L. Serrano, C.L. Ayala, S. Bruse, J.A. Tischfield.* Department of Genetics, Rutgers University, Piscataway, NJ.

Mitotic recombination in somatic cells is a main pathway to the loss of heterozygosity (LOH) that may lead to cancer if tumor suppressor genes are involved. In present study the C57BL6 x 129S2 F1 mice heterozygous for the adenine phosphoribosyltransferase (Aprt) gene were used as a model for studying in vivo mechanisms of LOH. In ear-derived fibroblasts of forty mice, 192 of 239 (80%) DAP-resistant clones were attributable to physical loss of the wild-type Aprt allele. 116 of these clones were genotyped with SSR markers located 1 - 73 cM along chromosome 8. After making the most conservative correction for possible sib clones, more than half of clones exhibited a breakpoint in the 47 - 67 cM interval. We selected nine informative SNPs located in this 20 cM interval. We genotyped with a method that utilizes multiplex PCR, ligation chemistry, FlexMap microspheres, and the Luminex100 flow cytometer for efficient and robust SNP genotyping. We found an elevated frequency of mitotic crossovers in two regions within the 47 - 67 cM chromosomal interval. 43% of 30 breakpoints occurred in the 6-Mbp interval between 113.1 Mbp - 120.8 Mbp, and 20% in the 2-Mbp interval from 106.1 Mbp - 108.1 Mbp. Both of these frequencies represent more than the expected number of crossovers than would be predicted for these intervals based on meiotic map distances or a random distribution of crossovers based on DNA length. The details of sequence analysis of these target regions will be presented.

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Simplified amplification approach for all exons and regions of interest for PTEN, ATBF1, and hMLH1/hMSH2 gene(s) followed by mutation detection using DHPLC. *B.L. Legendre[1], J. Zhu[1], G. Wu[1], J. Durocher[3], S. Lilleberg[2].* 1) Research & Development, Transgenomic, INC., Omaha, NE; 2) Translational and Clinical Research, Transgenomic, INC., Omaha, NE; 3) Translational and Clinical Research, Transgenomic, INC., Gaithersburg, MD.

Mutation detection by denaturing high performance liquid chromatography (DHPLC) has become a robust and accepted technology in screening for genetic variations, both germline and somatic, due to its high accuracy and sensitivity, high throughput, and low running costs. Screening of all exon sequences and other regions of interest of a particular gene for known and unknown mutations is important in the understanding of cancer development, treatment choices, treatment response and outcome monitoring. One current issue for mutation screening with DHPLC analysis is the lack of a simple, standardized protocol for the sample preparation and simultaneous amplification for all exons of the gene(s) of interest. In order to minimize the PCR amplification time and skill level needed, PCR-based 96-well plate assays were developed for numerous genes and their regions on interest which are prevalent in cancer screening, including PTEN (including the promoter region of exon 1, prostate cancer), ATBF1 (recently shown to be linked to prostate cancer), and hMLH1/hMSH2 (hereditary nonpolyposis colorectal cancer [HNPCC]). This technique involves identifying primers sets with similar annealing temperatures for all exons and regions of interest for a specific gene (PTEN, ATBF1) or set of genes which are associated with a particular disease (hMLH1/hMSH2 for HNPCC or ATBF1, PTEN, and hMLH1/hMSH2 for prostate cancer). These primers sets are then aliquoted into defined wells of the 96-well plates, allowed to dry, and then covered with a thin film. The plates are then stored at room temperature until use. In this study, we demonstrate the use of these gene specific (PTEN, ATBF1) or disease specific (hMLH1/hMSH2 for HNPCC) sample amplification assays followed by DHPLC analysis under partially denaturing conditions or DNASep-based HPLC analysis under nondenaturing conditions using SURVEYOR Nuclease.

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Molecular diagnosis of CML (RT- PCR for bcr-abl gene)compared with cytogenetic diagnosis. *Z. Ataei Kachoei[1], L. Andonian[2], MT. Akbari[3], MR. Khorramizadeh[4], MA. Patton[5], M. Hashemzadeh Chaloshtori[6].* 1) Dept of medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; 2) Dept of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 3) Dr Akbari Medical Genetic Lab, No 98, Taleghani Ave, Tehran, Iran; 4) Dept of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 5) Dept Medical Genetics , St Georges Hospital, Medical School, Cranmer Terrace, London, UK; 6) -Dept of Biochemistry and Genetics, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Chronic myelogenous leukemia (CML) is a clonal myeloprolifrative disorder of hematopoietic stem cells (HSC). The Pathologic hallmark of CML is the Philadelphia (ph) chromosome, which is a result of reciprocal translocation between long arms of chromosome 9 and 22 [t(9;22)(q34;q11)]. This translocation transposes Abl exon a2 into the M-bcr and in lees cases m-bcr-region so that hybrid gene has b3a2, b2a2 or e1a2 sequence. This study is a comparison between the results of molecular and cytogenetic tests for detection of ph chromosome for 29 patients in presentation of CML. Patients were referred to genetics laboratory by oncologists or hematologists according to the clinical diagnosis for CML and befor taking any drug. Altogether 23 patients were ph(+) by molecular test but 5 of them were cytogenetically ph(-). The result of both cytogenetic and molecular test for 2 cases was negative. Molecular study for 39 patients shows the different rate of b2a2 (7cases), b3a2 (9cases)and e1a2 (7 cases) fussions and coexpression of these fusious in some cases (b2a2 / b3a2 6 cases , e1a2 / b3a2 1case, b2a2 / b3a2 / e1a2 2 cases) .Odds ratio between molecular and cytogenetic test in this study is 1.3 . Keyword: CML , RT-PCR, Molecular diagnosis, Cytogenetic, Iran.

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Novel microarray-based resequencing using pyrophosphate-activated polymerization (PAP). *Q. Liu, S.S. Sommer.* Dept of Molecular Genetics, City of Hope Ntnl Med Ctr, Duarte, CA.

The rate and cost of DNA resequencing limit molecular epidemiological analysis and clinical molecular diagnosis. Pyrophosphate-activated polymerization (PAP) is a novel technology for nucleic acid amplification and DNA sequencing. In PAP, DNA polymerase serially couples pyrophosphorolysis and polymerization using a 3 blocked nucleotide (P*). This serial coupling exhibits a high selectivity to mismatches along the length of the blocked oligonucleotide. Herein, this property is used for a resequencing microarray technology with an unparalleled signal to noise ratio, in which P*s are anchored to a surface and processed in a high throughput manner. For each nucleotide on the template strand, four P*s, one wildtype and three single base substitutions, were synthesized with identical sequence complementary to the template except at the 3' terminus, where ddAMP, ddTMP, ddGMP or ddCMP correspond to the wild type sequence and the three possible single base substitutions. For resequencing, a 20 nucleotide region of the human factor IX gene, a total of eighty P*s were synthesized. Then each was anchored onto one spot of a CodeLink microarray through a 5 amino linker. DNA samples of wildtype, single base substitution, micro-deletion and micro-insertion were examined. All sequences were called correctly. Thus, we demonstrate the feasibility of a high throughput microarray-based resequencing for detection of unknown mutations.

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SCIMLA: Testing the lipophilic mutagen hypothesis of breast cancer (LMHOB). *E. Saleh, A. Bockholt, S.S. Sommer.* Dept Molecular Genetics, City of Hope, Duarte, CA.

Background:Breast cancer is the most frequent malignancy occurring in women. The origins of sporadic breast cancer are largely unknown. There is growing indirect evidence that mutagens in the environment and particularly of dietary origin may play a causative role in the etiology of breast cancer. Breast tissue has a unique architecture:small islands of cancer-prone cells surrounded by a sea of fat cells. Hypothesis:Breast tissue may be a preferential target for fat-soluble mutagens. Cancer-prone cells of breast tissue are hypothesized to be 1) particularly sensitive to fat-soluble mutagens originating in animal feed and 2) differentially susceptible to the type of fat consumed in the diet. In adulthood, adipocytes cannot replicate but they can concentrate the great diversity of lipophilic mutagens from meat products which derive from animal feed (lipophilic mutagen hypothesis of breast cancer LMHOB). We hypothesize that mammary mutation load (mutation frequency, pattern, and spectrum) differs dramatically among women, and that, the pattern of mutation in breast adipocytes and mammary cells correlate within an individual. Method:Single Cell Immunohistochemical Mutation Load Assay(SCIMLA)is a novel method to measure somatic mutation load in solid tissues. SCIMLA requires reporter genes in which missense mutations often cause protein accumulation. Single cells that are immunohistochemically positive for protein accumulation are microdissected from ethanol fixed and paraffin embedded tissues. The reporter gene is amplified from the single cell and sequenced for somatic mutation. Results:SCIMLA was used to test a first prediction of the LMHOB. Our observation of marked interindividual variation in mammary cell mutation pattern is consistent with LMHOB. Implications:The marked interindividual variation in mutation load among women requires explanation. Based on these data a mouse model is being used to examine directly the effect of dietary lipophilic mutagens on mutation frequency and pattern in mammary vs colon and lung epithelium. If LMHOB is correct monitoring the mutagen content of animal feed could be implemented to reduce the incidence of breast cancer.

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Mutation screening of UBE4A, a homologue of the putative neuroblastoma tumour suppressor gene UBE4B. *H. Caren, A. Holmstrand, R.M. Sjoberg, T. Martinsson.* Department of Clinical Genetic, Gothenburg University, Gothenburg, Sweden.

Chromosome 11q and 1p are commonly deleted in advanced stage neuroblastomas and are therefore implicated to contain tumour suppressor genes involved in the development of neuroblastoma. The two UFD2 homologs, UBE4A and UBE4B, involved in the ubiquitin/proteasome pathway, reside in 11q and 1p respectively. UBE4B has previously been analyzed for mutations and one mutation (a mutation in splice donor site of exon 9; c.1439+1G>C) was found in one neuroblastoma tumor with fatal outcome. We now speculated that the homolog UBE4A could be an alternative way to tumorgenesis. The coding exons of UBE4A were therefore sequenced. One putative missense mutation (a 1028T>C, leading to a putative missense mutation, p.I343T, residing in exon 8) was found in one neuroblastoma tumor 20R8 and this was confirmed by sequencing in both directions. The change, an isoleucine (unpolar) to threonine (polar) was situated in a highly conserved amio acid region. In adition two novel variants were also found in intronic sequences of UBE4A. Both the UBE4A and the UBE4B genes are implicated in the ubiquitin proteasome pathway by encoding proteins that are homologues of the yeast protein Ufd2. One could speculate that the proteins generated from UBE4B and UBE4A are involved in protecting the cell from environmental stress and that inactivation of either of them could contribute to malignancy.

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Mutation and LOH analyses of cell cycle genes in skin melanoma: cyclin D1, p53, p21, p16, p15, and Rb1. *J. Durocher, C. Wasserburger, J. Hempel, D. Foster, M.L. Nickerson, S.L. Lilleberg.* Translational and Clinical Res, Transgenomic, Omaha, NE.

The transition from phase G1 to S of the cell cycle is controlled by sequential activation of cyclin/Cdk complexes (Cyclin-dependent kinases). Active cyclin/Cdk complexes phosphorylate and inactivate members of the retinoblastoma protein (Rb) family, which are negative regulators of G1 and S-phase progression. This leads to the induction of E2F-regulated gene expression and cell proliferation. Cdk inhibitors directly bind to and interfere with cyclin/Cdk complexes, and negatively regulate cell cycle progression. The TP53 tumor suppressor gene and Cdk-inhibitors such as CDKN1A, CDKN2A, and CDKN2B are altered and thus aberrantly regulated in different types of tumors. In order to investigate the role of the genes involved in the control of G1/S phase cell cycle progression in human melanomas, we determined the spectrum of mutations in TP53, CCND1, CDKN1A (p21), CDKN2A (p16), CDKN2B (p15), and RB1 genes in primary and metastatic melanomas and melanoma cell lines. Sixty (60) melanomas and 12 melanoma cell-lines were analyzed utilizing an in-depth scanning strategy that consists of heteroduplex-based mutation detection by DHPLC and Surveyor Nuclease with the WAVE System. Results will be presented suggesting that these genes are involved in melanoma tumorigenesis, although at low frequencies. Other tumor suppressor genes that may be informative of the mechanism of tumorigenesis in skin melanomas will also be discussed.

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Mutations Screening in BRCA1 /2 Genes in Non -Ashkenazi Women. *A. Elimelech[1], M. Sagi[2], C. Shochat[1], S. Korem[1], D. Levi[1], L. Kaduri[2], A. Nissan[2], T. Peretz[2], D. Bercovich[1,3].* 1) human genetic, migal, kiryat -shmona, Israel; 2) Department of Human Genetics, Dept. of Surgery 2 and Department of Oncology, Hadassah University Hospital, Jerusalem; 3) Tel Hai Academic College, Israel.

Increased knowledge of breast cancer genetics has improved the possibilities to predict the risk for developing breast and ovarian cancer. In Israel, genetic testing and screening of women at high risk is done at present only for the founder mutations, according to the ethnic origin: in Ashkenazi -185delAG and 5382insC in the BRCA1 gene, and 6174delT in BRCA2, for Yemenite Jews- the mutation 8675delAG in BRCA2 and in Persian Jews - the 3053T>G in BRCA1. For other non-Ashkenazi Jewish women and for Arab women, no common mutations predisposing to breast cancer were found. Using DHPLC mutation detection technique we screened 12 Israeli Arabs or non-Ashkenazi Jewish women with breast cancer and significant family history and 6 controls, for mutations in the BRCA1/2 genes. Our goal was to screen the full sequences of the two genes in order to find all cancer causing mutations and/or sequence variations that could be modifiers alleles to breast cancer. Screening 31 fragments covering the 24 exons of the BRCA1 gene revealed three mutations, which can be suspected as deleterious. Of the three, one was found in non-Ashkenazi Jewish women, novel mutation P1812A in exon 23 (were also found in two affected sisters). Two mutations were found in Israeli Arabs: E1373X in exon12 and M1652I in exon16. The E1373X mutation was found in four affected family members. By screening 40 fragments covering the 27 exons of BRCA2, 3 mutation was found, one of them on a codon 1646del4 (5164del GAAA) in exon 11d, for a woman with no mutation in the BRCA1, which was previously reported (in the (Breast Cancer Information Core (BIC) -NHGRI). In the BRCA1gene, a total of 17 DNA alterations were found: 1 nonsense, 8 missense, 4 IVS, 1 5UTR and 3 silent. The complete sequence variations for each participant in both genes will be presented. Screening these mutations in large populations of non-Ashkenazi women can add private or common mutations in non-Ashkenazi Jews or Israeli Arabs, and will help developing new screening programs for these populations.

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***EGFR* Mutation Status and Pathological Features of Primary Lung Cancers.** *K.E. Finberg, J. Beheshti, E.J. Mark, A.J. Iafrate.* Department of Pathology, Massachusetts General Hospital, Boston, MA.

Somatic mutations in the kinase domain of the epidermal growth factor receptor (*EGFR*) have been detected in a subset of patients with pulmonary adenocarcinoma and correlate with clinical responsiveness to the tyrosine kinase inhibitors gefitinib and erlotinib. Here we correlate adenocarcinoma subtype and *EGFR* mutation status in 130 primary lung cancers. DNA was isolated from microdissected tissue, and the nucleotide sequence encoding the kinase domain (exons 18-24) of *EGFR* was analyzed by PCR and capillary gel electrophoresis. Heterozygous *EGFR* mutations predicted to alter the coding sequence were identified in 24/130 (18.5%) tumors. Of the previously reported mutations associated with clinical response to gefitinib, the 2573T>G mutation in exon 21 (resulting in L858R) was detected in 7 tumors, while 12 tumors harbored in-frame deletions within exon 19. 27/130 tumors analyzed were bronchioloalveolar carcinomas (BACs) or adenocarcinomas with bronchioloalveolar features; of these 27 tumors, *EGFR* mutations were detected in 9 tumors (33%), consistent with prior reports of a high prevalence of BAC among tumors with *EGFR* mutation. All of these 9 tumors were of the non-mucinous histologic subtype. In contrast, 10 of the 18 BACs or adenocarcinomas with bronchioloalveolar features that did not have *EGFR* mutation showed mucinous differentiation. Thus, in BACs or adenocarcinomas with bronchioloalveolar features, the presence of mucinous differentiation was a negative predictor of *EGFR* mutation (^{2}=5.74 with Yates' correction, df=1, p=0.02). There was no correlation between BAC histological subtype, or other adenocarcinoma histologic subtype, and specific *EGFR* mutations. Prior work has demonstrated a high frequency of *KRAS* mutation in mucinous-type BAC, and recent study has shown that lung tumors refractory to gefitinib or erlotinib often have *KRAS* mutations and that these tumors lack *EGFR* mutation. Thus, our data suggest the possibility that in BACs mucinous differentiation may correlate with refractoriness to EGFR kinase inhibitors.

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Ultra-Deep Sequencing of EGFR from Lung Carcinoma Patients Reveals Low Abundance Drug Response Mutations. *M. Egholm[1], E. Nickerson[1], R. Thomas[2], G. Turenchalk[1], B. Desany[1], M. Ronan[1], S. Hutchison[1], J. Knight[1], L. Du[1], J. Leamon[1], J.M. Rothberg[1], T. Tengs[2], J.F. Simons[1], M. Meyerson[2].* 1) 454 Life Sciences, Branford, CT; 2) Dana-Farber Cancer Institute, Boston, MA.

A current challenge in oncology is the detection of cancer causing and chemotherapy responsive mutations in clinical samples that may contain only a small sub-population of tumor-derived cells. Mutation detection by Sanger sequencing is typically restricted to mutations present at 20% abundance or greater.

Here we report high-sensitivity mutation detection using a new high-throughput DNA sequencing technology with the ability to simultaneously sequence 200,000-400,000 templates, in excess of 100 bases each, within 4 hours. The massive over-sampling enables mutation detection below 1% without *a priori* knowledge of the mutations. We have demonstrated the utility of the technology by studying mutations in the epidermal growth factor receptor (EGFR), associated with tumorigenesis and response to tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC). We examined the mutational status of exons 18-21 of EGFR in over twenty NSCLC patients. Overlapping PCR products covering the five target exons were simultaneously sequenced to 1000-fold coverage per template. Our data revealed low abundance mutations, including base-pair substitutions, deletions and insertions associated with disease and drug-response, not formerly detected with Sanger sequencing. In a pre-therapy pleural effusion sample from a patient with strong initial drug response, a drug-sensitizing 18 base-pairs deletion was detected at 0.28%. Sequencing of a post-therapy sample taken after relapse determined that the deletion was present at 2.5% and, consistent with the clinically observed loss of drug response, also revealed a drug-desensitizing mutation, T790M, at 2.0%.

Our data demonstrate fast and accurate identification of cancer-associated mutations from complex samples at a sensitivity and speed that is unprecedented. The rapid mutation detection can aid in critical decision making regarding course of treatment and potentially affect patient outcome.

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Cooperation between tumor suppressor genes: accelerated tumorigenesis in a child with constitutional mutations in the *p53* and *PTEN* genes. *S.E. Plon[1], H.V. Russell[1], J.G. Nuchtern[1], J.Y. Kim[1], M. Hegde[1], M.B. Bhattacharjee[1], J. Hicks[1], C. Eng[2], L.L. Wang[1].* 1) Depts of Pediatrics, Surgery, Pathology, and Molecular and Human Genetics, Baylor College Medicine, Houston, TX; 2) Human Cancer Genetics Program, The Ohio State University, Columbus OH.

Studies of sporadic cancers suggest that inactivation of *p53* and *PTEN* tumor suppressor genes are mutually exclusive events. However, mouse models heterozygous for both *p53* and *Pten* mutations demonstrate accelerated tumor development comparable to *p53* homozygous null mice, demonstrating that haploinsufficiency at both loci may cooperate in tumorigenesis. Here we describe a four-year-old girl who inherited a *PTEN* missense mutation, L112V, from her father and a *p53* hotspot mutation, R282W, from her mother. The paternal history is consistent with Cowden Syndrome (CS) with macrocephaly, lipomas and thyroid disease. The probands mother and sister, both heterozygous for the *p53* mutation, were diagnosed with cerebellar astrocytoma and medulloblastoma, respectively. The proband displays clinical features of CS including macrocephaly, multiple lipomas and hemangiomas. However, her tumor spectrum is not typical of either CS or Li Fraumeni syndrome and includes: metastatic neuroblastoma (*MYCN* non-amplified) at age 7 months, anaplastic juvenile granulosa cell tumor of the ovary at age 16 months, pleomorphic xanthoastrocytoma of the left temporal lobe at age 3 years, and liposarcoma of the pelvis, metastatic to bone and lung, at age 4 years. Cytogenetic analyses of the neuroblastoma, ovarian and brain tumors revealed different abnormalities including hyperdiploidy, acquisition of trisomy 21, and telomeric association of chromosome 15. Thus, the accelerated and unique pattern of tumors in this child suggests cooperation between the *p53* and *PTEN* gene products to maintain genomic stability. Studies to confirm this hypothesis are underway utilizing cell lines established from family members. More generally, studies of sporadic human tumors that focus on inactivation of both copies of a tumor suppressor gene may not appreciate the contribution of haploinsufficiency at two different tumor suppressor loci to tumorigenesis.

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MSI colorectal cancer is characterized by decreased expression of CDK2-AP1. *Z. Yuan[1], A. Bennett[2], T. Sotsky Kent[2], A. Miller[2], T.K. Weber[1, 2].* 1) Department of Molecular Genetic; 2) Surgery, Albert Einstein of College of Medicine,NY.

Background: We have demonstrated Cyclin Dependent Kinase2 -Associated Protein 1 (CDK2-AP1) expression is significantly decreased in microsatellite unstable (MSI) CRC cell lines (Yuan et al., 2003). Recently, we observed a novel del T alteration in a microsatellite-like sequence of CDK2-AP1 associated with its decreased expression in MSI CRC. We also observed siRNA inhibition of CDK2-AP1 expression associated with increased colon cell proliferation and decreased apoptosis (Yuan et al., 2005). In this study we: 1) Employed multiple MMR deficient systems to study MMR modulation of CDK2-AP1 expression. 2) Utilized MASS spectrometry analysis of purified CDK2-AP1 protein complexes to investigate the mechanism of CDK2-AP1 regulation of cell growth and apoptosis.**Materials and Methods**: mRNA and protein expression of CKD2-AP1 were determined in MSH2 knockout mouse embryonic stem (ES) cells vs MSH2 wild type mouse ES cells and a MLH1 knockout mouse vs a MLH1 wild type mouse using real-time RT-PCR and Western blot assays. CDK2-AP1 protein expression was analyzed in 50 human CRC tissues (43 MSS/7 MSI) using tissue microarray assays. CRC CDK2-AP1 protein complexes were purified using a tandem affinity purification (TAP) system. Purified CDK2-AP1 protein complexes were characterized using MASS Spectrometry. **Results**: CDK2-AP1 mRNA and protein expression were significantly decreased in MMR knockout mouse ES cells and mouse knockout models using real-time RT-PCR and Western blot assays. In tissue arrays, CDK2-AP1 expression was significantly decreased in 6/7 human MSI CRCs. Interestingly, 14 candidate proteins including cell growth and apoptosis control elements, were identified as components of the CDK2-AP1 protein-protein complex by MASS Spectrometry. **Conclusion**: These results support and expand our novel published observations of a relationship between MMR deficient CRC and decreased CDK2-AP1 expression. CDK2-AP1 protein complex analysis identified a number of candidate mediators of CDK2-AP1 modulation of cell growth and apoptosis. These results further support an important role for CDK2-AP1 in the initiation and progression of MMR deficient CRC.

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Altered Expression of the Early Mitotic Checkpoint Gene *CHFR* in Breast Cancers: Implications for Tumor Suppression. *L.M. Privette[1], M.E. Gonzalez[1], A.E. Erson[2], E.M. Petty[1].* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Biology, Middle East Technical University, Ankara, Turkey.

Introduction: Every three minutes, a woman in the United States is diagnosed with breast cancer. Many of the genetic changes that occur in breast cancer lead to defects in cell cycle checkpoint regulation. The early mitotic checkpoint gene *CHFR* (Checkpoint with FHA and Ring Finger) delays mitotic entry and aids in the survival of normal cells in response to microtubule stressors like nocodazole and paclitaxel. This suggests that CHFR expression may have prognostic value for cancer patients, yet little is known about this recently discovered gene.

Methods and Results: We analyzed Northern and Western blots to determine if CHFR expression was altered in breast cancer cell lines. Results indicated that CHFR mRNA and protein expression was lower in 12 of 25 cell lines when compared to immortalized mammary epithelial cells. Similar results were noted in ovarian cancer cell lines. When mammary epithelial cells expressing low levels of CHFR were retrovirally transduced with a *CHFR* construct, both an immortalized (HPV 4-12) and a cancer cell line (Hs578t) showed reduced growth kinetics and had a decrease in the mitotic index, with and without nocodazole treatment. Importantly, expressing *CHFR* in the breast cancer cell line, Hs578t, resulted in a dramatic five-fold decrease in invasion through a Matrigel matrix. Immunofluorescence also indicated that CHFR colocalizes with F-actin at the edge of the cytoskeleton. Preliminary tests to lower *CHFR* expression by RNAi indicate that epithelial cells have a higher mitotic index and also show some cytoskeletal defects.

Discussion: Together, these results suggest that low *CHFR* expression contributes to rapid growth and unregulated progression into mitosis whereas over-expressing *CHFR* lowers the growth rate and invasiveness of some breast cell lines. These results have implications for oncogenesis and begin to characterize *CHFR* as a tumor suppressor gene. Since *CHFR* expression is associated with a cellular response to paclitaxel, further characterization of *CHFR* could have great clinical impact.

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Analysis of a human septin gene, *SEPT9v1*, in mammary cells suggests novel oncogenic properties. *E.M. Petty, M.E. Gonzalez, L.M. Privette, E.A. Peterson, J.L. Loffreda-Wren.* Int Med/Hum Gen, Univ Michigan Med Ctr, Ann Arbor, MI.

Introduction: Fourteen human septin genes have been described in the past 10 years but their diverse functional roles await elucidation. Septins are members of an evolutionarily conserved GTP-binding family of proteins that localize to cytoskeletal filaments. They are implicated in cellular functions ranging from apoptosis to vesicle trafficking and associated with disorders ranging from Alzheimer's disease to cancer. We cloned *SEPT9* from a region of 17q25 allelic imbalance in cancer cells while others discovered it as an MLL fusion partner in leukemia cells. It is 96% identical to *Sept9/Sint1*, a proto-oncogene identified in murine lymphomas and mammary tumors. *SEPT9* is essential for normal cytokinesis.

Methods/Results: Upon finding *SEPT9* amplification and increased mRNA and protein expression of specific SEPT9 variants in the majority of breast cancer cells studied, we developed *in vitro* models to examine the consequences of *SEPT9* variant expression. Analysis of stable retrovirally transduced *SEPT9v1* expression in mammary epithelial cells revealed altered cell morphology, accelerated growth kinetics, enhanced foci formation, increased cell motility, higher invasive potential, dissociation of alpha-tubulin microfilaments, bi-nucleated giant cells, nuclear mis-localization, elevated mitotic indices, and significant aneuploidy when compared to control cells. While *SEPT9v2* and *SEPT9v4* variants also demonstrated high expression in some breast cancer cells, ectopic expression of *SEPT9v2* did not alter cellular phenotypes and *SEPT9v4* changes were less striking than those observed with *SEPT9v1*.

Discussion: Our data suggest that targeted expression of certain *SEPT9* variants, specifically *SEPT9v1*, may contribute to genomic instability and alter cellular proliferation, motility, and architecture, thus driving malignant progression in mammary epithelial cells. Further *SEPT9* analysis will deepen our understanding of novel septin-associated molecular pathways relevant to oncogenesis. Knowledge of *SEPT9's* functional roles in cancer may have prognostic and therapeutic implications.

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Somatic microindels. *S. Sommer[1], K. Gonzalez[1], K.A. Hill[1,2], K. Li[1], X. Li[1], W.A. Scaringe[1,3], J. Wang[1].* 1) Dept Molecular Genetics, City of Hope, Beckman Res Inst, Duarte, CA; 2) Dept of Biology, The University of Western Ontario, London, ON CANADA; 3) Bionformatics Group, Dept. of Molecular Genetics, City of Hope National Medical Center, Duarte, CA.

Microindels are infrequent mutations, often resulting in protein truncations. Little is known of the mutational mechanisms of microindels, and analyses are limited to the germline. Our database of 6,016 independent somatic mutations in Big Blue mice permits the first detailed analyses of 30 somatic microindels and direct comparisons with features of 795 pure microinsertions/microdeletions. Each microindel is unique. Microindel frequency shows no age, gender, or tissue specificity. Microindels have a similar frequency in mouse tumors and normal tissues, consistent with a similar endogenous contribution of microindels in cancer. 1-2 microindels (1 bp inserted and 2 bp deleted) are the most common. In microindels, deletions tend to be larger than insertions. The size distribution of deletions or insertions in microindels decays more slowly than for pure microinsertions/microdeletions. Many insertions in microindels do not repeat the adjacent nucleotides, while 97% of pure microinsertions do. Mouse somatic microindels and human germline microindels display many similarities, consistent with similar processes in mouse, humans, germline, and soma. Functionally, 13% of either somatic or germline microindels are in-frame, causing a minor change in protein size, which can play a crucial role in protein-tinkering in oncogenesis and evolution. Somatic microindels in Big Blue account for 17% of protein-tinkering expansions and 5% of protein-tinkering contractions. Analysis of somatic microindel sequences suggests heterogeneous mechanisms. Some seem to arise by error-prone processes such as the recruitment of error-prone polymerases at replication-blocking adducts. It is estimated that a human adult carries roughly 1.6 trillion somatic microindels (20% 1-2 microindels) with the potential to predispose to cancer.

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The human single-strand DNA binding protein RPA rapidly associates with DNA breaks in vivo. *I. Pasic[1,2], M.S. Meyn[1,2,3].* 1) Genetics & Genomic Biology, Hospital for Sick Children, Toronto, ON; 2) Molecular & Medical Genetics, Univ of Toronto, Toronto, ON; 3) Paediatrics, Univ of Toronto, Toronto, ON.

Human replication protein A (RPA) is a heterotrimeric single-stranded DNA-binding protein involved in DNA replication, recombination, and repair. In response to genotoxic insults such as -radiation, the 34kDa RPA2 subunit of RPA becomes phosphorylated at multiple serine and threonine residues. This response is, in part, dependent on the DNA damage response kinases ATM and DNA-PKcs. Phosphorylation promotes interactions between RPA and DNA repair proteins and shifts RPA away from a role in DNA replication. However, it is unclear whether RPA phosphorylation affects its ability to interact with damaged DNA.

To answer this question and better understand the role of RPA in the initial response to DSBs, we studied the recruitment of endogenous RPA and GFP-tagged RPA2 to the sites of laser microbeam-induced double-strand DNA breaks (DSBs) in human fibroblast nuclei. We can detect DSB-associated RPA foci 15 minutes and 1 hour post-irradiation. In contrast to ?-H2AX, RPA foci are small and punctate, reflecting tight spatial localization of RPA with damaged DNA.

The initial association of GFP-tagged wild type RPA2 with DSBs can be detected <2 seconds post-irradiation. This association is largely unchanged by RNAi-mediated depletion of DNA-PKcs in wild type or ATM-deficient human cells. However, the proportion of nuclei with damage-induced RPA foci 15 and 60 minutes post-irradiation is reduced in ATM-deficient fibroblasts and by specific RNAi-mediated depletion of DNA-PKcs in wild type and ATM-deficient fibroblasts. In addition, we find that the association of GFP- RPA2 with DSBs is decreased in NBS1-deficient human fibroblasts. Taken together, these findings implicate DNA damage kinases and the MRE11/NBS1/RAD50 complex in the interaction of RPA with DSBs and suggest that phosphorylation is required for the persistent association of RPA with damaged DNA but not for RPAs initial interactions with DSBs.

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DOES BRCA2 HETEROZYGOSITY RESULT IN HAPLOINSUFICIENCY IN HUMAN BREAST CELLS? *R. Bruchim, L. Quenneville, C.L. Panasci, W. Foulkes, R.S. Aloyz.* LDI. JGH, Montreal, qc, Canada.

Heterozygous carriers of mutations in the BRCA2 gene have a high risk of developing breast cancer. In these individuals, BRCA2 appears to act as a tumor suppressor gene, in that loss of the wild type allele (LOH) is frequently observed within tumors, leading to loss of BRCA2 function. BRCA2 protein has a critical role in DNA repair via homologous recombination and cytokinesis. We established a breast epithelial cell line (B28) derived from tumor tissue of a woman who developed an invasive ductal breast cancer and carries the truncating mutation BRCA2:5578delAA. Sequence analysis of the tumour shows only the mutated allele, whereas B28 retains heterozygosity at BRCA2. The mutation results in a truncated BRCA2 protein lacking the Rad51 association domain and the nuclear localization signal. Consistently, full length BRCA2 is detected in B28 cells and BRCA2 and Rad51 foci are detected in these cells after Cisplatin treatment. The cytokeratin (CK) expression profile of B28 cells suggests that the cells display a basal epithelial phenotype. Briefly, all the cells expressed HMW keratins (1, 5, 10 and 14), EGFR and IGF-1R. Moreover, 60% of the cells expressed CK 8/18, CK5/6 and p63. No staining for ER/PR, CK19 and SMA was detected. Additionally, 5-10% of the cells were positive for vimentin. In the present communication we report that B28 cells are more sensitivity than HNME cells and CAPAN-1 (human pancreatic deficient BRCA2 cell line) to Cisplatin, Etoposide and Taxol. Interestingly late passages of B28 cells are more sensitive to DNA damaging agents at early passages than at late passages. In contrast there is not significant difference in the sensitivity to Taxol between early and late passages of B28 cells. To our knowledge this is the first report suggesting that BRCA2 heterozygous cells display a phenotype regarding the sensitivity to chemotherapeutic agents. This is probably due to the fact that lymphocytes from carrier patients or engineered mouse cells have been utilized to assess BRCA2 haploinsuficiency in heterozygous cells.

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The BARD1 Cys557Ser variant confers susceptibility to breast cancer in carriers of BRCA2 999del5 and increases risk for multiple primary breast tumors. *S.N. Stacey[1], P. Sulem[1], A. Helgason[1], J. Gudmundsson[1], O.T. Johannsson[2], J.T. Bergthorsson[1], L. Amundadottir[1], U. Thorsteinsdottir[1], A. Kong[1], J. Gulcher[1], K. Stefansson[1].* 1) deCODE Genetics, Reykjavik, Iceland; 2) Dept of Oncology, LSH, Reykjavik, Iceland.

BRCA1 protein exists predominantly as heterodimers with a related protein, BARD1. Some pathogenic BRCA1 mutations disrupt the function of the BRCA1/BARD1 complex. Recently a missense BARD1 variant, Cys557Ser, was reported at increased frequencies in breast cancer (BC) families. We investigated the BARD1 Cys557Ser variant in a population-based sample of 1232 Icelandic BC patients and 703 controls. We then used the Icelandic national genealogy to study the relationships between the Cys557Ser variant and familial clustering of BC. Cys557Ser was present at an allelic frequency of 0.025 in BC probands unselected for family history and 0.016 in controls (risk ratio= 1.61, p=0.036). The frequency was 0.034 (risk ratio= 2.24, p=0.018) in a high-predisposition group of probands defined by family history, early onset and multiple incidences of primary BC. The frequency of the variant allele amongst carriers of the common Icelandic BRCA2 999del5 mutation in the high-predisposition group was 0.059 (risk ratio= 3.93, p=0.034). In contrast to BRCA2 999del5 carriers, BARD1 Cys557Ser carriers did not drive familial clustering of BC on their own. However, Cys557Ser carriers were at a more than 2-fold risk of subsequent primary breast tumors after the first BC diagnosis. Lobular and Medullary BC were over-represented in Cys557Ser carriers. An excess of ancestors of contemporary carriers lived in a single county in the southeast of Iceland. Most carriers shared a microsatellite haplotype suggestive of a founder event. The variant occurred on a single SNP haplotype background in the HapMap CEPH sample of Utah residents, suggesting a single ancient origin for common forms of the variant. Our findings suggest that BARD1 Cys557Ser is an ancient pathogenic variant that can confer risk for single and multiple primary breast cancers in combination with other known or unknown predisposition genes.

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Associations between the Glutathione S-Transferase Pi (*GSTP1*) Ile104Val polymorphism and lung cancer: a meta-analysis of published studies. *C.S. Hoffman, C.L. Avery, K.E. North, D.A. Canos, A.F. Olshan, C. Poole.*
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Lung cancer is the leading cause of cancer-related deaths in the US and has been directly associated with cumulative smoking exposure. The detoxification of smoke in the lung is a multi-step process in which the GST family of proteins, particularly *GSTP1*, plays a role. Multiple studies have examined the association between the Ile104Val (A>G) variant and lung cancer. A systematic review and meta-analysis was conducted to describe the state of the literature, with the goal of informing decision making and future research. We examined evidence of publication bias, evaluated the consistency of results overall and with regard to study characteristics, and estimated summary estimates when warranted. Published studies were obtained from Pubmed, OMIM, Biosys, and ISI Web of Science between May, 2004-June, 2005 and supplemented with citations from retrieved articles. Results were abstracted by two independent extractors and unadjusted odds ratios (OR) were calculated for three genotype contrasts (AG, GG, and AG/GG versus AA). We evaluated a dominance effect (AG/GG versus AA), as estimated odds ratios for this contrast were available for all 22 studies. There was evidence of funnel plot asymmetry (log rank test p=0.05, regression test p=0.2) and the trim and fill analysis imputed five hypothetically unpublished results. This reduced both the homogeneity test p-value (0.02 to <0.001) and the random-effects summary odds ratio estimate (OR = 1.11 to OR = 1.02). Funnel plot symmetry test p-values were higher within geographic strata, with OR estimates of 1.09 (95% CI: 0.97, 1.22) and 1.02 (95% CI: 0.91, 1.14) in the European and North American strata, respectively. Studies conducted in Asia produced higher and more heterogeneous odds ratio estimates than studies conducted elsewhere and did not support a summary measure. There was not compelling evidence of a consistently strong effect of the *GSTP1* polymorphism on lung cancer risk. Our future work will involve analyses that distinguish between one and two copies of the G allele and the evaluation of interaction by smoking.

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Variable expression and activity of taxane-metabolizing enzymes in ovarian tumors as a potential mechansim of taxane resistance. *J. DeLoia[1], W. Zamboni[2], J. Jones[1].* 1) Dept OB/GYN/RS, Univ Pittsburgh, Pittsburgh, PA; 2) Molecular Therapeutics and Drug Discovery Program, UPCI, Pittsburgh, PA.

Objective: Current firstline chemotherapy for ovarian cancer consists of carboplatin combined with either paclitaxel or docetaxel. Disposition of carboplatin is determined by renal clearance, while the taxanes are metabolized by cytochrome P450 (CYP450) enzymes. Although the majority of taxane metabolism occurs in the liver, recent data has shown that some solid tumors express CYP450 enzymes in the tumors themselves. The objective of this study was to determine whether ovarian tumors express genes regulating cellular efflux and subsequent metabolism, and whether histology, grade or stage correlated with expression. Methods: Gene expression of CYP2C8, CYP3A4/A5 and the ABC transporter ABCB1 was determined in 56 primary epithelial ovarian tumors. Cells were grown from seven tumors and exposed ex vivo to paclitaxel (PAC) and docetaxel (DOC) for up to 24 hours. PAC and DOC concentrations were measured in the media by an LC-MS assay. Results: Results from this analysis demonstrate that ovarian cancer cells do express functional taxane-metabolizing enzymes. Such expression appeared to enhance the ability of cancer cells to metabolize DOC. Specifically, the PK of DOC was correlated with the ratio of CYP4A5 to ABCB1 gene expression, thus representing a novel mechanism of chemotherapy resistance. There was no relationship between PAC PK parameters and gene expression. Unlike hepatic expression, these genes were not induced by exposure to drug. Conclusions: Knowledge of inter-individual variation in CYP450 enzyme tumor expression and activity may influence the individualization of chemotherapy, by avoiding agents that are rapidly metabolized and selecting agents that are not.

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Association of polymorphisms of dopamine receptor/transporter genes with risk of non-small cell lung cancer. *D. Campa[1,2], S. Zienolddiny[1,3], R. Barale[2], A. Haugen[3], F. Canzian[1].* 1) International Agency for Research on Cancer, 69372 Lyon Cedex 08, France; 2) Department of Science for the Study of Man and Environment, University of Pisa, Pisa, Italy; 3) Department of Toxicology, National Institute of Occupational Health, P.O. Box 8149 Dep., 0033 Oslo, Norway.

Tobacco smoking is the most important cause of lung cancer, however less than 20% of smokers develop lung cancer, suggesting that genetic factors play a role in the etiology of lung cancer. Dopamine receptor/transporter genes may be of interest in assessing NSCLC risk .Dopamine has been shown to inhibit the growth of several types of malignant tumors in mice, and this activity has been variously attributed to inhibition of tumor-cell proliferation and stimulation of tumor immunity. In addition, the dopamine concentration in malignant tumors is reported to be significantly reduced compared with that of normal control. Moreover, the neurotransmitter dopamine inhibits angiogenesis induced by VPF/VEGF. Angiogensis has an essential role in many important pathological settings including tumors. Dopamine strongly and selectively inhibits the vascular permeabilizing and angiogenic activities of VPF/VEGF. Dopamine exerted this role through dopamine receptors, inducing endocytosis of VEGF receptor 2, which is critical for promoting angiogenesis, thereby preventing VPF/VEGF binding, receptor phosphorylation and subsequent signaling steps. To evaluate the lung cancer genetic susceptibility we used an oligonucleotide microarray based on the arrayed primer extension (APEX) technique, The APEX technique consists of a sequencing reaction primed by an oligonucleotide anchored with its 5' end to a glass slide and terminating one nucleotide before the polymorphic site. The extension with one fluorescently labeled dideoxynucleotide complementary to the template reveals the polymorphism. A total of 26 oligonucleotides were designed to flank 13 SNPs of DRD2, DRD4 and DAT1 genes. A case-control study, on a total of 861 individuals of norwegian origin, showed that a 2 to 4-fold increased lung cancer risk is associated with DRD2, DRD4 and DAT1 genes
Keywords:dopamine lung cancer, SNPs.

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Association of MLH1 and MSH2 Polymorphisms and Colorectal Cancer Risk: A Population-Based, Case-Control Study of Canadian CRC Cases. *M. Mrkonjic[1], S. Raptis[1], J. Knight[1], D. Daftary[1], B. Younghusband[2], S. Gallinger[1], R. Green[2], J. McLaughlin[1], B. Bapat[1].* 1) Samuel Lunenfeld Research Inst, University of Toronto, Toronto, Ontario, Canada; 2) Memorial University, St.John's, NFLD, Canada.

Colorectal cancer (CRC) is a complex disease attributed to the interplay between genetic and environmental risk factors. The most commonly inherited form of CRC is hereditary nonpolyposis colorectal cancer (HNPCC); an autosomal dominant syndrome marked by tumors expressing genome-wide microsatellite instability (MSI) and caused by germline mutations in the mismatch repair (MMR) genes. Mutations in two MMR genes, MLH1 and MSH2, account for ~85% of cases among confirmed HNPCC patients. A number of single nucleotide polymorphisms (SNPs) in MMR genes have been identified, but little is known about the role of most of these SNPs in CRC. We hypothesize: A) that some MMR SNPs affect functional variation in MMR and contribute to CRC susceptibility and B) that the role specific MMR SNPs play in the development of CRC may vary by population. Specifically, a founder population with a high incidence of CRC, such as Newfoundland, may have a limited set of SNPs, which will have a greater population attributable risk compared to a more diverse population such as Ontario. We performed a case-control study and looked at associations between candidate SNPs and family history, MSI status, age of onset and location of the CRC tumors. The analysis was performed using lymphocyte DNA isolated from over 1000 CRC cases and 1000 controls from Ontario, as well as 500 cases and 350 controls from Newfoundland. Selected SNPs were genotyped using TaqMan assay. Statistical analyses of the selected MLH1 SNPs- I219V (p=0.73), -93G>A (p=0.17), IVS14-19A>G (p=0.70) indicated no significant association with the overall incidence of CRC. IVS14-19, however, was confirmed to be in linkage disequilibrium with I219V. MSH2 SNP G322D was significantly associated with family risk among CRC cases (p=0.02). Analysis of MSH2 promoter SNP -118T>C is currently in progress. Characterization of such SNPs in MMR genes will help to enhance our understanding of CRC development.

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Localization of Breast Cancer Susceptibility Loci by Genome-Wide SNP Linkage Disequilibrium Mapping. *T. Kirchhoff, N.A. Ellis, N. Mitra, T. Ye, S. Chuai, H. Huang, K. Nafa, K. Offit.* Dept Medicine, Mem Sloan Kettering Cancer Ctr, New York, NY.

Five to 15% of incident breast cancer (BC) cases and only half of the hereditary BC cases result from mutations in putative cancer susceptibility genes such as *BRCA1* and *BRCA2*. Attempts to map other BC susceptibility genes by linkage analysis have been hampered by genetic heterogeneity, decreased penetrance, and chance clustering. We are utilizing an association approach to localize BC genes, using genome-wide panels of single nucleotide polymorphisms (SNPs) and taking advantage of the genetic enrichment of susceptibility alleles in high-risk cases. Our ascertainment was focused on the Ashkenazi Jewish (AJ) population because it was established from a small number of founders and consequently the regions of increased linkage disequilibrium (LD) flanking the founder mutations are usually large (>1 million bases). With Affymetrix GeneChip arrays, we genotyped 8,487 SNPs in 3 sets of AJ BC cases: a validation set of 27 BC cases, all of whom carried the *BRCA2*6174delT* founder mutation, a field set of 19 BC cases from male BC kindreds, which simulated conditions for finding new genes, and a test set of 57 probands from BC kindreds (4 or more cases/kindred), in which mutations in *BRCA1* and *BRCA2* had been excluded. To identify associations, we compared the frequency of genotypes and haplotypes, respectively, in cases versus controls by the Fishers exact test and a maximum likelihood ratio test. In the validation set, we demonstrated the presence of a region of LD on *BRCA2*6174delT* chromosomes that spanned over 5 million bases. In the field set, we showed that this large region of LD flanking *BRCA2* was detectable despite the presence of heterogeneity in the sample set. Finally, in the test set, two regions of increased interest ($p<10e-5$) were identified on chromosomes 15 [TSC582885-TSC948257] and 9 [TSC181069-TSC1555460], and a third region ($p<10e-3$) emerged that had been identified previously by linkage analysis on chromosome 8. These results demonstrate the feasibility of the genome-wide association design and provide insights into optimizing its application.

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Identifying Associations Between SNPs In Cell Cycle Control Genes And Susceptibility To Ovarian Cancer. *S. Gayther[1], H. Song[2], S. Ramus[1], R. DiCioccio[3], L. Quaye[1], E. Hogdall[4], C. Hogdall[4], I. Jacobs[1], D. Easton[2], B. Ponder[2], A. Whittemore[5], S. Kruger Kjaer[4], P. Pharoah[2].* 1) University College London, UK; 2) University of Cambridge, UK; 3) Roswell Park Cancer Institute, Buffalo, USA; 4) Institute of Cancer Epidemiology, Danish Cancer Society, Denmark,; 5) Stanford University School of Medicine, USA.

BRCA1 and BRCA2 are responsible for the majority of families containing multiple cases of epithelial ovarian cancer. However, high-risk susceptibility genes are responsible for less than 30% of the excess familial ovarian cancer risks, which suggests other ovarian cancer susceptibility genes exist. The remaining risk may due to combinations of several common alleles of moderate/low risk, rather than other rare high-risk genes. We have used a SNP tagging candidate gene approach to look for associations between single nucleotide polymorphisms (SNPs) in three ovarian cancer case-control studies from Denmark, the UK and USA. These studies comprise ~1,500 cases and 2,500 controls. We chose to look for disease associations in genes involved in cell cycle control because there is extensive evidence for a ubiquitous role of cell cycle genes (e.g. p53, RB, p16) in cancer development generally and ovarian cancer specifically. So far we have genotyped 51 SNPs in 13 genes (CCND1, CCND2, CCND3, CCNE1, CDK2, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, RB1, p53). Genotype distributions were close to those expected under Hardy-Weinberg equilibrium. Genotype frequencies in cases and controls, stratified by study, were compared using a likelihood ratio test in a logistic regression model. For 46 SNPs, we detected no significant differences in genotype frequency between cases and controls. Two associations with $P < 0.05$ and three associations with $P < 0.01$ were seen for SNPs in CCND1, CDK6 and CDKN1B. In summary, we have found evidence that common variants in cell-cycle control genes are associated with increased ovarian cancer risk. In order to validate the role of these genes in ovarian cancer, it will first be necessary to confirm these results in even larger series of ovarian cancer cases and to identify the causative functional variants.

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***CHEK2* I157T associates with colorectal cancer risk.** *O. Kilpivaara[1], P. Alhopuro[2], P. Vahteristo[2], L.A. Aaltonen[2], H. Nevanlinna[1].* 1) Dept OB/GYN, Helsinki Univ Ctr Hosp, Helsinki, Finland; 2) Dept Medical Genetics, University of Helsinki, Helsinki, Finland.

CHEK2 is a serine/threonine kinase which takes part in various cellular functions regulating cell cycle progression, apoptosis, and DNA damage repair. CHEK2 is activated in response to DNA double strand breaks and it then phosphorylates various substrates including p53, BRCA1, and CDC25A. *CHEK2* germline mutations have been identified, first in breast cancer patients, and then also in patients with other types of cancer. The two most studied variants, 1100delC and I157T show statistically significant associations with breast and prostate cancer. Very recently, it was suggested that *CHEK2* I157T variant confers an elevated risk also for colon, kidney, and thyroid cancers as well as for non-Hodgkin lymphoma (Cybulski C. *et al.* AJHG, 2004). We have here studied the effect of *CHEK2* I157T on colorectal cancer predisposition in Finland. A total of 973 colorectal cancer patient samples were successfully analyzed for *CHEK2* I157T variant. The mutation frequency among population-based series of colorectal cancer cases was 7.8% (76/973), which is significantly higher than in Finnish population controls (5.3%), OR=1.5, 95% CI 1.1-2.1, p=0.008). Furthermore, the carrier frequency among familial colorectal cancer cases was 10.4% (14/135 OR=1.5, 95% CI =1.1-3.7 p=0.01). Our results are in agreement with the previously observed association between *CHEK2* I157T and colon cancer and suggest that *CHEK2* I157T is a risk allele for colorectal cancer in the Finnish population.

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Germline Mutations in the PTEN promoter affect autoregulation of its expression through interaction between PTEN and p53. *Y. Tang*[2,3,4], *C. Eng*[1,2,3,4,5,6,7]. 1) Clinical Cancer Genetics Program; 2) Human Cancer Genetics Program; 3) Comprehensive Cancer Center; 4) Department of Molecular Virology, Immunology and Medical Genetics; 5) Division of Human Genetics; 6) Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, USA; 7) Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, Cambridge CB2 2YZ, UK.

PTEN is a tumor suppressor with dual phosphatase activity and mutations of its gene, PTEN, have been associated with many sporadic cancers and heritable neoplasia syndromes including Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS). In the present study, we demonstrate that cell lines from CS and BRRS patients with germline PTEN promoter mutations in the vicinity of the p53 binding motifs have altered p53 regulation. This appears to be due to reduced PTEN stability and decreased PTEN-p53 interactions. By utilizing luciferase reporter assay and ectopic expression system, we demonstrate that PTEN is able to autoregulate its expression through the stabilization of p53. We further show that PTEN enhances p53 transactivation, a relationship which requires the interaction between PTEN and p53, and which is PTEN phosphatase-independent. Our data provide clues to better understand the regulation of PTEN expression and the possible mechanisms of the pathogenesis of the subset of CS individuals with germline promoter variation and who lack mutations in the PTEN coding region and splice sites. This mechanism also holds for those sporadic tumors that lack intragenic mutations but have hemizygous deletion of PTEN, which includes the promoter region as manifested by loss-of-heterozygosity of 10q markers.

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EGFR Mutations in Japanese lung cancer patients: A retrospective study of 198 cases and examination of a novel therapeutic application using siRNA targeting _EGFR_. *S. Yamanaka[1,2], M. Sato[2], R. Fujisaki[1], A. Sakukrada[2], T. Kondo[2], A. Horii[1].* 1) Dept Molecular Pathology, Tohoku Univ School of Med, Sendai, Japan; 2) Department of Thoracic Surgery, Institute of Development, Aging, and Cancer, Tohoku University.

We collected 198 surgically resected lung cancers and analyzed mutations in *EGFR*, *KRAS*, *TP53*, and *ERBB2*. Eighteen of them had been treated with gefitinib. Mutations in *EGFR* were found in 35 cases; all of them were adenocarcinoma (33%; 35 of 106 cases with adenocarcinoma), and females accounted for 74%. No tumors harbored mutations in both the *EGFR* and *KRAS* genes, but 10 of the 35 tumors harbored both *EGFR* and *TP53*. *ERBB2* mutations were less frequent. Mutations of *EGFR* were well associated with a positive response to gefitinib; all the patients with *EGFR* mutations except two cases without lung lesions showed clinical PR. We then performed in vitro studies to validate the gefitinib treatment for lung adenocarcinoma. Cells with *EGFR* mutations were very sensitive to gefitinib, and those with *KRAS* mutations were quite resistant. We further examined siRNA-mediated knockdown of *EGFR* and found that the effects were quite similar to those of gefitinib. Our present results imply that (i) mutation analyses of *EGFR* and *KRAS* provide valuable information about whether or not apply treatments targeting against EGFR and the selection of dosage for such treatments, and (ii) siRNA-mediated knockdown may be effective in treating cases of acquired resistance to gefitinib.

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Cytologic, molecular and proteomic analysis of breast ductal fluid from *BRCA1* and *BRCA2* gene mutation carriers and controls. *I. Locke[1,2], Z. Kote-Jarai[1], E. Bancroft[2], M. Le Bihan[3], P. Osin[1,2], A. Nerurkar[1,2], C. Isacke[1], G. Gui[1,2], L. Izatt[4], G. Pichert[4], R. Eeles[1,2], The Carrier Clinic Collaborators.* 1) Institute of Cancer Research, Sutton, UK; 2) Royal Marsden Hospital, London, UK; 3) St George's Hospital Medical Biomics Centre, London, UK; 4) Guy's Hospital, London, UK.

Aim: To evaluate the ductal approach for risk assessment in *BRCA1/2* families. Methods: We recruited 53 women from *BRCA1/2* families (21 *BRCA1*, 20 *BRCA2* and 12 non-carriers) for nipple aspirate fluid (NAF) collection from their healthy breasts. Ductal lavage (DL) was attempted in a subgroup of 35 women. Two slides, produced using the Shandon cytospin technique, were Giemsa-stained for cytologic assessment. Free DNA was extracted from the DL supernatant (DLS) using a DNeasy Tissue Kit (Qiagen). Loss of heterozygosity (LOH) analysis of free DNA from 34 ducts was performed by fluorescence-based PCR amplification of 7 *BRCA1/2* microsatellite markers. The methylation status of a panel of genes was investigated using a multiplexed methylation-specific PCR technique. Proteomic profiling of NAF and serum from carriers and controls was performed on three array surfaces and analysed by SELDI-TOF mass spectrometry with data interpretation using Biomarker Wizard software (Ciphergen). Results and Conclusions: NAF was obtained from 30% *BRCA1* carriers, 45% *BRCA2* and 30% of controls. At least one breast duct was cannulated in 80% of NAF producers but only 27% of non-NAF producers. Overall 65% of cytology slides were deemed adequate for diagnosis. Two *BRCA* carriers were found to have epithelial cell atypia (C3) in the absence of clinical or radiological findings suspicious of malignancy. No controls thus far have had atypia. We found high levels of LOH, but reproducibility was not consistent. *Cyclin D2* and *RAR* genes were found to be uniformly unmethylated. Cu-IMAC30 array surfaces were found to give the most data-rich proteomic spectra for NAF. Peaks discriminating healthy carrier and control breasts were found and are being evaluated as biomarkers of risk.

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Associations with family history of cancer in a population survey. *M. Scheuner[1], P. Yoon[1], S. Coughlin[2], T. McNeel[3], N. Breen[4], A. Freedman[4].* 1) Office of Genomics and Disease Prevention, CDC; 2) Division of Cancer Control and Prevention, CDC; 3) IMS, Inc; 4) Division of Cancer Control and Population Sciences, NCI.

Few population-based studies have investigated associations between personal and family history of cancer. Using the 2001 California Health Interview Survey data, we describe associations between cancer family histories and breast, prostate and colorectal cancer. Of the 55,513 respondents, 34.4% reported a cancer family history. Personal history of any cancer was reported by 8%; 0.5% had colorectal, 2.4% of females had breast, and 1.5% of males had prostate cancer. Odds ratios were calculated by comparing cancer family histories for respondents with and without breast, prostate and colorectal cancer, adjusting for demographic factors. Family history of breast cancer, and family history of ovarian cancer were associated with breast cancer (OR=2.0, 1.6-2.5 and OR=1.8, 1.1-3.1, respectively). Associations with breast cancer were higher when family histories included breast and ovarian (OR=4.7, 2.1-10.4), breast and melanoma (OR=2.3, 1.1-5.0) or breast and prostate cancer (OR=2.6, 1.5-4.4). Family history of prostate cancer was associated with prostate cancer (OR=3.2, 2.1-4.8). Stronger associations with prostate cancer were observed when family histories included ovarian and melanoma (OR=25.4, 4.1-158), breast and melanoma (OR=14.8, 4.0-55), breast and ovarian (OR=9.7, 2.4-39) or prostate and colorectal cancer (OR=4.5, 1.3-15.6). Family history of colorectal cancer was associated with colorectal cancer (OR=2.3, 1.5-3.4), and associations were also found given family histories of ovarian and cervix (OR=14.9, 7.2-31.2) or colorectal and breast cancer (OR=2.5, 1.1-5.4). Our results show similar associations to those found in case-control studies with a 2 to 3-fold increase in breast, prostate and colorectal cancer given a family history. This suggests population-based, self-reported data are useful in assessing familial risk. Certain combinations of cancer family histories display associations of similar or greater magnitude. Recognizing these familial patterns may have implications for risk assessment.

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MyGenerations:A computerized family history risk assessment tool for the public. *S.M. O'Neill, W.S. Rubinstein.*
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Family history is a risk factor for many chronic diseases, but systematic collection of family history is not practiced routinely in health care settings. When collected, the information is often not interpreted, and management is not targeted to calculated risk levels. Integrated tools that assess risk based on family history data, and other personal risk factors, could be of value to clinicians, patients, and researchers. We have developed an interactive touchscreen computer program called MyGenerations which obtains family cancer history and performs breast cancer risk assessments. The program produces a printed pedigree, calculated risks for breast cancer and BRCA mutations, and risk-tailored prevention/screening messages. We are currently adding risk assessment algorithms for several other cancer syndromes. MyGenerations kiosks are installed in our hospital lobby, cancer center lobby, and mammography suite. In the first 11 months, 737 users tried the program. Of the 577 users (78%) who continued past the login screens, 344 (47%) completed all segments of the program. The average time for completion was 9.5 minutes (range 2 to 78). In this population, 12% had some form of cancer, but BRCAPRO mutation risk was generally low. Only 1.2% were classified as high risk (>8%) and 2.3% as moderate risk (3% -8%). However, among unaffected women, 23% had a Gail 5-yr risk above the 1.67% threshold. Survey questions at the end were answered by 280 people (81% response rate). Most found the program easy to use (88%) and said they would definitely recommend it to friends (74%). While 66% said they would definitely show their printed family history tree to their family, only 39% said they would show it to their physician. We designed the program so users could remain anonymous. Only 33 (9%) of the users who completed the program chose to provide identifying contact information. MyGenerations is well accepted by users and enables them to record their own family history of disease, learn about their risks, and receive information about actions to take. However, further research is needed to determine the barriers to sharing the information with physicians.

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Evaluation of *RAD50* in Familial Breast Cancer Predisposition. *J. Tommiska[1], S. Seal[2], A. Renwick[2], R. Barfoot[2], L. Baskcomb[2], H. Jayatilake[2], J. Bartkova[3], J. Tallila[1], M. Kaare[1], A. Tamminen[1], P. Heikkilä[4], D.G. Evans[5], D. Eccles[6], K. Aittomäki[7], C. Blomqvist[8], J. Bartek[3], M.R. Stratton[2,9], H. Nevanlinna[1], N. Rahman[2], Breast Cancer Susceptibility Collaboration (UK).* 1) Dept. Obstetrics and Gynecology, HUCH, Helsinki, Finland; 2) Institute of Cancer Research, Sutton, UK; 3) Danish Cancer Society, Copenhagen, Denmark; 4) Dept. Pathology, HUCH, Finland; 5) Dept. Medical Genetics, St Marys Hospital, Manchester, UK; 6) Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton; 7) Dept. Clinical Genetics, HUCH, Finland; 8) Dept. Oncology, HUCH, Finland; 9) Cancer Genome Project, The Wellcome Trust Genome Campus, Hinxton, Cambs, UK.

The genes predisposing to familial breast cancer are largely unknown, but five of the six known genes are involved in DNA damage repair. RAD50 is part of a highly conserved complex important in recognising and repairing DNA double-strand breaks. Recently, a truncating mutation in the *RAD50* gene, 687delT, was identified in two Finnish breast cancer families. To evaluate the contribution of *RAD50* to familial breast cancer we screened the whole coding region for mutations in 435 UK and 46 Finnish familial breast cancer cases. We identified one truncating mutation, Q350X, in one UK family. We screened a further 544 Finnish familial breast cancer cases and 560 controls for the 687delT mutation, which was present in 3 cases (0.5%) and 1 control (0.2%). Neither Q350X nor 687delT segregated with cancer in the families in which they were identified. Functional analyses suggested that *RAD50* 687delT is a null allele as there was no detectable expression of the mutant protein. However, the wild-type allele was retained and expressed in breast tumors from mutation carriers. The abundance of the full-length RAD50 protein was reduced in carrier lymphoblastoid cells, suggesting a possible haploinsufficiency mechanism. These data indicate that *RAD50* mutations are rare in familial breast cancer and either carry no, or a very small, increased risk of cancer. Altogether, these results suggest *RAD50* can only be making a very minor contribution to familial breast cancer predisposition in UK and Finland.

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DC-SIGN Haplotypes are Associated with Risk of Prostate Cancer in Afro-Caribbeans of Tobago. *Y. Li[1], J. Martinson[2], C.H. Bunker[3], A.L. Patrick[4], R.E. Ferrell[1].* 1) Human Genetics; 2) Infectious Diseases and Microbiology; 3) Epidemiology, University of Pittsburgh, Graduate School of Public Health; 4) Tobago Regional Hospital, Scarborough, Tobago.

Epidemiology and genetic epidemiology studies suggest a role for chronic inflammation in the etiology of prostate cancer. Several candidate prostate cancer susceptibility genes (MSR1, RNASEL, TLRs) are involved in innate immunity and inflammation. DC-SIGN is a promiscuous C-type lectin receptor, expressed on macrophages, dendritic cells and B cells of the innate immune system, which capture and process foreign antigens for presentation to the adaptive arm of the immune system. To assess the impact of DC-SIGN variation on prostate cancer risk in the high risk population of Tobago, we report an exhaustive interrogation of the DC-SIGN promoter region in a case/control study. Cases and controls were identified through a population based screening study in Tobago men, ages 40-79 years. 1.8 Kb of the proximal 5'-flanking region of DC-SIGN was resequenced in 153 cases and 172 controls. Haplotypes were estimated using the PHASE software package. Association was assessed by chi square analysis. We identified 26 single nucleotide polymorphisms, six of which had a minor allele frequency >7.5% (-2267G>A, -1567A>T,-1326C>T,-336C>T, -201C>A, -139T>C). Six site haplotypes were estimated and there was significant evidence of association (p=0.01) between haplotypes and prostate cancer. Haplotype GATTCC was more frequent in controls (17% vs. 2%) and haplotype GACTCC was more prevalent in cases (17% vs. 7%). This association was not explained by any single SNP. The association of haplotypes at the DC-SIGN locus and prevalent prostate cancer suggests a role for infection in the etiology of prostate cancer in the high risk population of Tobago.

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Prevention of lung adenocarcinoma induced by benzo[a]pyrene in GNMT transgenic mice. *Y.M. Chen[1], S.P. Liu[1], J.H. Hung[1], F.Y. Li[2], T.F. Tsai[3].* 1) Inst Public Health; 2) Department of Pathology; 3) Department of Life Science, School of Life Science, Natl Yang-Ming Univ, Taipei, Taiwan.

Glycine N-methyltransferase (GNMT), also known as a 4S-polycyclic aromatic hydrocarbon (PAH)-binding protein, is a protein with multiple functions which including a) catalyzing glycine to sarcosine in the one-carbon metabolism pathway; b) affecting DNA methylation level through regulating the concentration of S-adenosylmethionine (SAM), a general methyl group donor for DNA MTs ; c) serving as a binding for Benzo[a]pyrene (BaP), preventing the BaP-DNA-adducts formation by BaP. We used mouse phosphoenolpyuvate carboxykinase promoter to establish a human GNMT transgenic (TG) mouse model to study the interaction between GNMT and BaP. The GNMT-TG mice were bred with HBV large surface antigen transgenic (LS-TG) mice to generate the following three groups: A, GNMT-TG mice; B, GNMT-LS double TG mice; and C, wild type (Wt) mice. Male mice from three groups were challenged with BaP (375£gg/7g body weight) intraperitoneally. Seventy-eight weeks after the challenge, the mice were sacrificed. In terms of lung tumor, the results showed that 3/4 mice of group C developed lung adenocarcinoma, but only 2/4 mice of groups A and 1/6 of B developed lung adenocarcinoma. Therefore, there were only two nodules in group A and B, respectively, but 9 nodules developed in group C. It indicated that over-expression GNMT in liver may play a protection role for lung tumor formation. This study is helpful for elucidating the role of GNMT in the tumorigenesis of lung which has important implications in the development of strategies for the prevention, diagnosis and treatment of lung cancer.

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Increased Tumor Frequency in Individuals with Isolated Hemihyperplasia and Molecular Alterations at 11p15.

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Isolated hemihyperplasia (IH) refers to asymmetric overgrowth of single or multiple organs or regions of the body. Recent reports document molecular alterations in 11p15 in individuals with IH, including paternal uniparental disomy (UPD) [Shuman et al., 2002; Grundy et al., 1991] and more recently methylation alterations at KCNQ1OT1 and H19 [Martin et al., 2005] but they do not report on the frequency of tumors in molecular subtypes of IH. We report here on the frequency of tumors in 51 patients with IH. Blood samples and when available skin and/or tumor tissue were obtained. Molecular alterations were detected at 11p15 in 11/51 patients: 8 (16%) with UPD, 3 (6%) hypomethylation at KCNQ1OT1 and 0 with hypermethylation at H19. Of the 8 patients with UPD, 4 had tumors (3 hepatoblastoma, 1 Wilms tumor); 0/3 patient with hypomethylation at KCNQ1OT1 had a tumor. Of the remaining 40 with no molecular alterations, 6 had tumors (3 Wilms tumor, 2 neuroblastoma, 1 adrenocortical adenoma). The 50% frequency of tumor development in patients with IH and UPD was significantly higher than the 15% frequency in those with IH and no molecular alteration detected (Fishers exact test p = 0.047, OR 5.67). Of note, the high overall frequency of tumors in the study population (19.6%) likely reflects a bias in ascertainment of patients with IH being referred for genetic testing. Our data demonstrate that patients with IH and tumors have molecular alterations known to increase tumor risk and highlight the importance of implementing molecular testing and tumor surveillance for all patients with IH.

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Severe Pfeiffer syndrome (PS) and Antley-Bixler syndrome (ABS) : Clinical and molecular evidences for differential diagnosis. *M. Gonzales[1], S. Heuertz[2], J. Martinovic[3], S. Delahaye[1], P. Loget[4], M. Le Merrer[2], A. Verloes[5], A. Bazin[3], J. Bonaventure[2].* 1) Fetal Pathology Unit, Hosp Saint Antoine, Paris, France; 2) INSERM U393, Necker Hospital, Paris, France; 3) Laboratoire Pasteur-Cerba, Cergy-Pontoise, France; 4) Centre Hospitalier du Mans, Le Mans, France; 5) Clinical Genetics Unit, Genetic Dept, and INSERM U676, Robert Debre Hospital, Paris, France.

PS and ABS are rare skeletal disorders characterized by severe craniofacial malformations and limb abnormalities. While PS is an AD disease, the mode of inheritance of ABS, originally described as AD, has been challenged, when a heterozygous mutation in the FGFR2 gene was reported to produce an ABS-like phenotype, suggesting that ABS could be genetically heterogeneous. We present here a series of 5 fetuses obtained after TOP following ultrasound detection of skeletal anomalies suggestive of severe non syndromic craniosynostosis. Clinical and radiological examination of fetuses following interruption revealed craniosynostosis with midface hypoplasia, frontal bossing, proptosis and variable anomalies of the axial and appendicular skeleton evocative of severe PS or ABS. Two of these patients had dysplastic ears, slender fingers with arachnodactyly, marked femoral bowing and radio-humeral synostosis. No FGFR2 mutations were identified in these cases but homozygous and compound heterozygous mutations in the cytochrome P450 oxydoreductase (POR) gene were found, supporting the clinical diagnosis of ABS. Out of the three other foetuses, two had elbow ankylosis and none of them exhibited femoral incurvation. Broad halluces were visible in two cases in the absence of arachnodactyly. All three foetuses had tracheal cartilaginous sleeve and fusions of cervical and/or lumbar vertebrae. The recurrent S351C FGFR2 mutation was detected in all three cases. Based on these findings, we suggest that severe PS would be the most likely diagnosis in these patients. The absence of tracheal cartilaginous sleeve and vertebral defects in patients carrying POR mutations indicate that these clinical signs are cardinal for the differential diagnosis of severe PS versus ABS.

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Update on the dup(22q) syndrome and expansion of the clinical phenotype. *S.J. Hassed, J. Lee, S. Li, J.R. Seely.*
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Since our first description of the dup(22q) syndrome in 2002 in a family with a relatively mild phenotype, we and others have reported several patients and families with this duplication. As more individuals with the dup(22q) are reported, the clinical phenotype has expanded. This condition is expected to be complimentary to the 22q deletion syndrome that encompasses the conditions previously referred to as velocardiofacial syndrome (VCFS), di George syndrome, and Shprintzen syndrome. The dup(22q) may be as common as the deletion, but recognized less frequently due to a less well defined phenotype and the standard laboratory technique of analyzing metaphase cells. It has been suggested that all FISH studies be evaluated by scanning of interphase cells in addition to the usual metaphase cells, since duplications are much less obvious on metaphase spreads. It is expected that, as this change in laboratory protocol becomes standard, the dup(22q) syndrome phenotype and incidence will be elucidated. We report an 11-month-old male with infantile spasms beginning at five months. EEG was consistent with a seizure disorder, but not characteristic for hypsarrhythmia. Normal studies included MRI, chromosome analysis (46,XY), amino and organic acids, and ophthalmological consult. Prior to the onset of seizures, development was reportedly normal. Psychomotor development has slowed markedly. Seizures are controlled with Klonopin. The mother reportedly had seizures beginning at 18 months that resolved. There are minor dysmorphic features, which resemble the mother. Two males on the maternal side of the family are reported to have congenital heart defects and learning disabilities. FISH analysis of 22q revealed ish dup(22)(q11.2q11.2)(TUPLE1X3) for the patient. FISH analyses on the family are pending. We suggest further expansion of the 22q duplication syndrome to include infantile spasms.

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Raising Awareness About Birt-Hogg-Dube Syndrome. *D. Kostiner, C. Miranda, A. Holt.* Dept. of Medical Genetics, Kaiser Permanente PC, Portland, OR.

Birt-Hogg-Dube syndrome (BHD) is an autosomal dominant condition that predisposes to fibrofolliculomas (small, firm, dome-shaped, papules), trichodiscomas (tumors of the hair discs), and acrochordons (skin tags). Associated findings can include spontaneous pneumothoraces, lung cysts, colonic polyps, and renal tumors. Features typically present in adulthood and show variable expression. The condition is likely underdiagnosed since most physicians have not heard of it and since many patients do not come to dermatologic attention. Molecular testing can detect 80% of mutations. We present the cases of two patients to raise awareness of BHD since the pulmonary, renal, and colonic manifestations can cause morbidity and mortality. Pt. #1 is a 53-year-old man with multiple fibrofolliculomas confirmed by biopsy, several flat skin tags, and hyperkeratosis of the hair follicles. The lesions first appeared in his 30s. Screening CXR and sigmoidoscopy were normal. His father had fibrofolliculomas and benign renal cysts and his PGM had fibrofolliculomas. He was offered molecular testing for BHD, but declined for fear of insurance discrimination. Pt. #2 is a 57-year-old man with multiple fibrofolliculomas confirmed by biopsy, unilateral pneumothorax at age 23, and right renal hypodensity found on screening CT. His mother had characteristic skin changes and a pneumothorax in her 50s. Molecular testing showed a deleterious duplication in exon 9 of the BHD gene. Follow-up care will include a repeat renal CT and screening colonoscopy. Discussion: Although patients are bothered by the skin findings of BHD for cosmetic reasons, the internal manifestations are far more dangerous. We recommend screening CXR, colonoscopy, and renal scan at time of diagnosis and periodically thereafter, and offering of molecular testing to at-risk relatives when the probands mutation is known. Educating primary care physicians about BHD might increase referrals to dermatologists, and thus increase overall rates of diagnosis.

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VACTER ASSOCIATION. *C. Martinez-Cruz[1,2], A. Garcia-Huerta[3], M. Diaz-Garcia[3], N. Lopez-Trinidad[3], G. Garcia-Sanchez[3].* 1) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 2) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 3) Servicio de Genética.Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación.México, D.F. e-mail gsanchezg03@yahoo.com.mx.

VATER is a mnemonically useful acronym for vertebral defects, anal atresia, tracheoesophageal fistula with esophageal atresia, and radial dysplasia. This combination of associated defects was pointed out by Quan and Smith (1972) Nearly all cases have been sporadic, with no recognized teratogen or chromosomal abnormality. VACTER association features overlapping those of Townes-Brocks syndrome and branchiootorenal syndrome. Case report. We report a Mexican male patient 14 -year- old with VACTER association. The parents of the patient are not consanguineous and the family history was negative for VACTER association. At 15 days of age he had undergone surgery for imperforate anus, he has bilateral radial hypoplasia, a bifid thumb on the right and preaxial polydactyly on the left side. Echocardiography at 6-month- old showed coarctation of the aorta. Left small, dysplastic ear, hearing impairment onset before 2 years old. The audiogram at 14 years old shows sensorineural profound hearing impairment. X-rays revealed Klippel-Feil type II (Vertebral fusion of C2-C3 and C5-C6). He has gastroesofagic reflux and mental retardation. Nowadays he is becoming aggressive. The kidneys were normal on ultrasound. Genetic molecular exam is in process.

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150 Patients with Facio auriculo vertebral spectrum. *A. Garcia-Huerta[1], L.M Rosales-Olivares[2], M. Diaz-Garcia[1], N. Lopez-Trinidad[1], C.F. Martinez-Cruz[3,4], R. Baez-Reyes[5], G. Garcia-Sanchez[1].* 1) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación. México, D.F; 2) Servicio de Cirugía de Columna, Centro Nacional de Rehabilitación/Ortopedia. Mexico, D.F; 3) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 4) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 5) Servicio de Genetica,Departamento de Investigacion. Instituto Nacional de Perinatologia, Mexico, D.F.E mail gsanchezg03@yahoo.com.mx.

Facio auriculo vertebral spectrum (FAV) is a disorder with variable expression that mainly involves structures developed from first and second branchial arches as well as ocular, vertebral, cardiac, renal and neurological anomalies. FAV has a heterogenous etiology: vascular disruption, lack of interaction of mesenchyme with neural crest cells and teratogens, among others. Some chromosomopathies have been described in patients with FAV, and recently a MSX gen mutation is suggested. This study with 150 patients with FAV shows the clinical findings: microtia: 99%, meatal atresia: 78.7%, facial asymmetry: l4% preauricular tags; l2.7% and submucous cleft palate:l6%. We also found cardiac anomalies in l.2%. No patient had ocular, renal or neurological anomalies, but vertebral malformations were present in l3.9%. Our patients had severe hearing loss: 59.4%, mainly conductive type; 64.3%. In 150 patients with FAV: 61% were sporadic cases. In 26%, they had recessive pattern of inheritance and 13% were dominant. We discuss the high frequency of adverse factors during early pregnancy: hemorrhages, medical administration or infections. The impact in reproductive conduct in parents of a child with FAV and the importance of searching minimum expression of FAV in all the family members.

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CHARGE association. Mother and daugther. *N. López Trinidad[1], C.F. Martinez-Cruz[2,3], M. Diaz-Garcia[1], A. Garcia-Huerta[1], G. Garcia-Sanchez[1].* 1) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación. México, D.F; 2) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 3) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México. E-mail gsanchezg03@yahoo.com.mx.

CHARGE association is characterized by coloboma, it is bilateral or unilateral, affecting the iris, retina and/or disc. Visual loss varies according to size and location of the coloboma. Heart defects are conotruncal anomaly, aortic arch anomaly, isolated septal defects are uncommon. Choanal atresia is bilateral or unilateral. Retarded growth and development. The diagnosis of mental retardation must be made with caution in anyone who has impaired hearing and/or vision. Hearing loss may cause language delay, while blindness impairs motor skills and genital hypoplasia. Although the most consistent features are still those prefixed bye the letters CHARGE, additional abnormalities frequently occur, these include facial palsy, renal abnormalities, orofacial clefts and tracheoesophageal fistulas. This association overlaps some features with Schprintzen and Cat eye syndrome. We report a female 17 years old patient with bilateral hearing loss, present at birth, language and psychomotor delayed. She shows short stature, facial asymmetry, hypertelorism, small auricles and nose and flat philtrum, absent uvula, thoracic asymmetry, scoliosis, multiple nevus, cardiac murmur, cubitus valgus, hypoplasic phalanges of thumbs. She also had swallow impairment. She had undergone a palatoplasty at 2 years old and fundoplasty at 3 years old. Her mother had facial asymmetry, scoliosis; small auricles, broad thumbs. We did not find any ocular anomalies on the patient or her mother. We discuss the minimum expression of CHARGE association in the mothers patient.

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Osteogenesis imperfecta type I. *S. Juarez-Garcia[1], L. Hernandez-Gomez[2], C.F. Martinez-Cruz[3,4], R. Baez-Reyes[5], G. Garcia-Sanchez[6].* 1) Instituto de la Comunicación Servicio de Neuropsicologia Infantil.Humana/Instituto Nacional de Rehabilitación; 2) Instituto de la Comunicación Servicio de Audiologia. Humana/InstitutoNacional de Rehabilitación; 3) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 4) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 5) Departamento de genetica.Instituto Nacional de Perinatologia, Mexico. D.F E; 6) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/InstitutoNacional de Rehabilitación.E. mail gsanchezg03@yahoo.com.mx.

Osteogenesis imperfecta (OI), often referred to as "brittle bone disease", is a heritable disorder characterized in most instances by either qualitative or quantitative abnormalities in the synthesis of the most important component of the bone matrix, type I collagen. Sillence in 1979 proposed a classification based on clinical, radiographic and genetic criteria, that quickly was adopted worldwide: Type I is the mildest form, with occasional fractures before puberty, minimal deformity and normal stature. Large families from that group were used to demonstrate linkage with COLlAl and COLlA2. In type II, the most severe (lethal) form, fractures in utero lead to pulmonary insufficiency and perinatal death. In type III, frequent fractures causing progressive deformities, short stature, and triangular face are characteristic. In type IV, deformities and dwarfism are present, but are less severe.We report a female patient 39-year-old, right hearing loss anset at 15-year-, left hearing loss onset at 20-year-old with slowly progression.Tinnitus alltime, Several bone fractures in the infancy. Exposition to noisy environment for 9 years, chicken pox and measles in the infancy.The pedigree shows two brothers with osteogenesis imperfecta. Physical exam: Prognatism, eyes with blue sclera. Opalescent teeth. Audiogram: Mixed severe bilateral hearing loss. Timpanogram: curves As of Jerger bilateral. Stapedial reflex: absent bilateral response.Caloric test with bilateral hyperreflexia.

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Wildervanck syndrome. Report of three Mexican patients. *M. Diaz-Garcia[1], C.F. Martinez-Cruz[2,3], A. García Huerta[1], R. Baez-Reyes[4], N. Lopez-Trinidad[1], G. Garcia-Sanchez[1].* 1) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación. México, D.F; 2) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 3) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 4) Servicio de Genetica, Departamento de Investigacion.Instituto Nacional de Perinatologia, Mexico, D.F. E-mail gsanchezg03@yahoo.com.mx.

The Wildervanck syndrome is a rare disorder characterized by a triad of features including congenital deafness (sensorineural, conductive, or mixed); fused cervical vertebrae (Klippel-Feil anomaly),and abduction and/or adduction (Duane syndrome). Occasional abnormalities include mental retardation, growth deficiency, cleft palate, ear abnormalities, etc. This syndrome may be responsible for at least 1%; of deafness among females; the deafness has been shown by radiologic studies to be due to a bony malformation of the inner ear and/or anomalies of the ossicular chain. The etiology is unknown. The disorder is almost limited, to females, raising the question of sex-linked dominance with lethality in the hemizygous male. We present three cases of Wildervanck syndrome. Patient 1: a 7-year-old male child with right hemifacial microsomia, right microtia, low occipital hairline. Bilateral abduction and adduction normal (Duane type I). Nystagmus, uvula bifida, submucous cleft palate and short neck. Audiometric study showed, sensorineural hearing loss, bilateral and profound. Ear CT scan, revealed Mondini malformations. Radiography of spine showed a congenital fusion of C2 and C3 vertebrae, thoracic hemivertebrae, scoliosis, and spina bifida occulta (Klippel-Feil type III). MRI of cervical spine showed continuity of the vertebral body of C2 and C3. Patient 2: a 16-year-old female with low occipital hairline; abduction and adduction, bilateral (Duane type 3). Low-set ears, mandible with left hypoplasia, broad short neck. Audiometric testing showed sensorineural hearing loss, bilateral and profound. Ear CT scan, revealed a Mondini dysplasia. Radiography study reveled fusion of C2-C7 vertebrae, scoliosis of C6 to C7 and alteration on T2. Patient 3: is a 10-year-old female with unilateral abduction on the right eye (Duane type I). Low-set ears and hypoplasics helix, hypoplasica uvula, short broad neck with limited move; probable spina bifida occulta. Radiography study will be discussed. The audiogram showed sensorineural hearing loss, bilateral and profound.

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Waardenburg syndrome: Differentiation between types and risks of deafness. *E. Pardono, J.F. Mazzeu, K. Lezirovitz, R.M.P. Nascimento, M.T.B.M. Auricchio, R.C. Mingroni-Netto, P.A. Otto.* CEGH, Depto de Genética e Biologia Evolutiva, IB-Universidade de São Paulo,Brazil.

The Waardenburg syndrome (WS) is clinically characterized by craniofacial dysmorphims, pigmentation defects (irides, skin and hair) and congenital sensorineural deafness. Since the penetrance rate of the telecanthus trait in WSI is high but incomplete, the clinical diagnosis of the variant (WSI or WSII) in isolated affected individuals without telecanthus is troublesome. We present the results of a study performed in 41 patients with WSI, 33 having the type II and 18 of them being isolated cases without telecanthus. From all these patients, 17 craniofacial measurements were obtained. We developed also a method that enables the estimation of frequencies of the eight cardinal signs used for the diagnosis of the condition, based on a total sample (containing our and literature cases) of 502 affected individuals with WSI and 154 with WSII and derived discriminant functions to separate individuals affected by one of the two variants. Discriminant analysis based on the frequencies of the eight cardinal signs can improve the correct separation of WSI patients without telecanthus from those presenting WSII: deafness is the most important characteristics for the diagnosis of the WSII in patients without telecanthus;the presence of defects of fusion of the medial region of the face (synophrys, nasal root hyperplasia, and lower lacrimal dystopia) favors the hypothesis of WSI. The discriminant function based on four ocular measurements (inner and outer intercanthal, interpupillary and lower interlacrimal distances), combined to four other measurements (nose interalar distance, mean length and width of ear, face height), perfectly classifies patients affected by one of the WS variants. We prepared also a table for use in the genetic counseling of WSI and WSII, that lists the risks of deafness for the offspring of carriers of the two conditions. Two new mutations in the PAX3 gene were found in ten, investigated propositi with WSI: the substitution c.142 G>T on exon 2 (48G>C) and the deletion c.764-776del(TTACCCTGACATT) on exon 5. Financially supported by CNPq/PRONEX and CEPID/FAPESP .

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Groll-Hirschowitz Syndrome.Two unrelated Mexican families. *G. Garcia-Sanchez[1], M. Diaz-Garcia[1], A. Garcia-Huerta[1], N. Lopez-Trinidad[1], C.F. Martinez-Cruz[2,3].* 1) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación. México, D.F; 2) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 3) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México. E-mail:gsanchezg03@yahooo.com.mx.

Hirschowitz et al. (1972) described 3 sisters, from a sibship of 6, who had progressive nerve deafness beginning at ages 8, 3 and 9 years and becoming complete or nearly complete by ages 10, 5 and 18 years, respectively. Vestibular function remained normal. Progressive sensory neuropathy without peripheral trophic changes was also present. Tachycardia and loss of the carotid sinus reflex may indicate involvement of the cardiac vagus. Involvement of the vagus nerve led to progressive loss of gastric motility. Two of the sisters were demonstrated to have multiple diverticula with jejunoileal ulceration from which the eldest sister died at age 18 years. Malabsorption of fat and intestinal loss of serum protein occurred. A surviving sister had marked acanthosis nigricans. Potasman et al. (1985) reported 2 sisters with the same or a similar disorder. The parents were first cousins. Peripheral nerve biopsy showed demyelinization. The patients died at ages 31 and 20 years, their disorder having manifested itself at age 24 and 13 years, respectively. We report two unrelated families with two sisters affected in each one. First family: The eldest maternal half sister apparently hearing, because her language development was normal, since 18 months of age, when she died due to abdominal pain, vomiting and diarrhea. The youngest maternal half-sister referred with hearing impairment, apparently prelingual. At 7 months of age she had undergone surgery for abdominal pain, vomiting and diarrhea. After surgery she is still vomiting and she has abdominal pain sometimes. The audiogram at 3 years old shows sensorineural bilateral profound hearing impairment. Second family: The eldest sister was referred as deaf-mute, died at 14 years old by abdominal pain. The youngest sister is referred with hearing impairment apparently prelingual and at 2 years old had undergone surgery for strong abdominal pain. Audiogram at 6 years old shows sensorineural bilateral profound hearing impairment. All patients reported with this syndrome and our patients are female. It is an X dominant linked syndrome?

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Evaluating the frequency that babies with *GJB2*-related hearing loss pass newborn hearing screening. *C.G. Palmer[1], A. Martinez[1], M. Telatar[1], N. Shapiro[1], M. Fox[1], B. Crandall[1], Y. Sininger[1], W.W. Grody[1], L.A. Schimmenti[2].* 1) UCLA, Los Angeles, CA; 2) U of Minnesota, Minneapolis, MN.

GJB2/GJB6 hearing loss variants are a common cause of mild to profound nonsyndromic sensorineural hearing loss (NSNHL). There currently is limited knowledge on the postnatal and early infancy period of *GJB2*-related hearing loss, raising questions about the age of identification of babies with *GJB2*-related hearing loss vis a vis newborn hearing screening (NHS). We present results on 63 infants/toddlers with apparent NSNHL from a longitudinal study in which NHS, audiology, and *GJB2/GJB6* status are analyzed. Genomic DNA was collected from the infants by buccal brush and subjected to complete *GJB2* sequencing and assay of del(*GJB6*-D13S1830). 19 infants (30%) were identified as having two *GJB2* or a combination of *GJB2/GJB6* hearing loss variants, and 2 of these infants (10.5%) passed NHS. 44 infants had 0 or 1 *GJB2* hearing loss variant, and 4 (9%) passed NHS. There was no significant relationship between NHS result and presence of 2 or 0/1 *GJB2/GJB6* hearing loss variants (F.E. p=1.0). Of the 2 infants with *GJB2*-related hearing loss who passed NHS, one has V37I/V37I genotype and was diagnosed at 21m with mild-mod sloping SNHL and the other has 35delG/167delT genotype and was diagnosed at 2m with profound SNHL. Of the 4 infants with non-*GJB2*-related hearing loss who passed NHS, 2 are heterozygous for 35delG (data available on one showed profound SNHL), one has V27I polymorphism and severe-profound SNHL, and one has no variants and a unilateral severe SNHL. These results suggest that newborns with two *GJB2* hearing loss variants are as likely to pass NHS as those with 0 or 1 variant, however larger samples are needed to confirm this finding. These results also suggest that *GJB2*-related hearing loss may have a variable age of identification, either due to a mild congenital phenotype that is more likely to be missed by NHS, a progressive phenotype, or a postnatal onset. Genetic studies in conjunction with NHS can provide the empirical data needed for elucidating the postnatal and early infancy detection period of *GJB2*-related hearing loss and for determining the timing of genetic testing.

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Distribution of mutations associated with sensorineural hearing loss in a minority population. *J. Neil[1], J. Lu[2], S. DiMauro[2], S. Shanske[2], B. Morrow[3], A. Shanske[1].* 1) Center for Craniofacial Disorders, Childrens Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, New York; 2) Laboratory of Molecular Neurogenetics, Columbia University, New York, New York; 3) Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York.

Approximately 50% of sensorineural hearing loss (SNHL) is attributed to genetic causes, the majority being non-syndromic. Mutations in many autosomal loci and several in mitochondrial DNA (mtDNA) have been identified. The mutation spectrum is known to vary by population, though little is known about the distribution in minority populations, including African Americans and Hispanics. Fifty charts of patients with non-syndromic SNHL referred to The Craniofacial Center were reviewed. Data regarding ethnicity, GJB2 (connexin-26) studies, mtDNA analysis (nucleotides 1555, 7445 and 3243, and the 12S rRNA gene) and CT scans of the temporal bones were collected. Fifteen of 48 patients tested for connexin-26 mutations were heterozygous for a known autosomal recessive mutation (35delG common mutation, 167delT or T101C), a benign polymorphism (G79A) or indeterminate changes (A503G, A670C or G380A). No patient had the A1555G or T7445C mtDNA mutations that have been associated with SNHL. Of the 31 patients with CT scan results, two had enlarged vestibular aqueducts, one of whom has a G79A polymorphism. Distribution by ethnicity identified one of nine American Blacks with G79A; one of eight Dominicans with A670C; two of two Guyanese with G380A; one Pakistani from a consanguineous family with G380A; two of nine Puerto Ricans with A503G (one also had an mtDNA mutation in the 12S rRNA gene, C1465T), a father and son with 35delG, one with G79A; and two of two Mexicans with G79A. A child with both 35delG and 167delT was of Brazilian, Jewish and Irish descent and an individual with T101C was of Sicilian, Italian and Irish descent. Our data suggest that specific mutation frequencies may vary by population. The high frequency of indeterminate nucleotide changes suggests that some may be pathogenic. We plan to validate these observations in a large prospective population study. We can then begin to correlate genotype with phenotype.

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Hearing Loss in the Genetics Clinic. *E. Prijoles, R. Gallagher, M. Manning, L. Hudgins.* Dept of Pediatrics, Div of Medical Genetics, Stanford University, Stanford, CA.

With the advent of the newborn hearing screen, more children with congenital hearing loss are identified, and an underlying etiology is sought. We report our experience in evaluating patients with hearing loss and provide recommendations. Chart review of 76 patients referred for hearing loss was performed; those with known diagnoses were excluded. Thirty (39%) were nonsyndromic and 46 (61%) were syndromic. Of the nonsyndromic patients, 20 had connexin 26 testing: 3 (15%) had 2 disease-causing mutations, 6 (30%) had one mutation, and 4 (20%) had recognized polymorphisms. A connexin 30 deletion was found in 1 of 5 patients tested. Four chromosome aberrations were detected: del 18q (2), dup 4q35 (1), and 13;14 translocation (1). A mother and son with features suggestive of an ectodermal dysplasia had a dominant connexin 26 mutation consistent with KID syndrome. Five had genetic testing for Pendred due to inner ear malformations on CT; all were negative. LT1 and LT5 for Jervell and Lange-Nielsen were also normal in an individual with prolonged QT. Other diagnoses included: peroxisomal disorders(2), BOR syndrome(2), Usher syndrome(1), Charcot-Marie-Tooth disease(1), and isolated unilateral developmental defect of the inner ear(1). Identifying an etiology of hearing loss can be challenging. A definitive diagnosis was made in only 20% of our patients. The paucity of definitive diagnoses may be due to ascertainment bias, e.g. those with obvious diagnoses were referred with a specific diagnosis (and excluded) or not referred at all. In summary: 1) Individuals with hearing loss and developmental delay should have a chromosome analysis, 2) Testing for del 18q should be performed in patients with small auditory canals, 3) Consider peroxisomal disorders in patients with seizures, and 4) A good clinical evaluation is still the best tool in directing other studies such as renal ultrasound and EYA1 for BOR, ERG for Usher, and CMT testing for those with an abnormal neurological exam. Hopefully, genetic testing for hearing loss will continue to improve, so we can offer more accurate prognostic information and recurrence risk counseling in the future.

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DNA sequence variations in *GJB2*, encoding Connexin 26: extreme allelic heterogeneity, variable carrier rates, and ethnic stratification of alleles. *R. Alford[1], HY. Tang[1], P. Fang[2], P. Ward[2], E. Schmitt[2], S. Manolidis[3], J. Oghalai[1], B. Roa[2].* 1) Otorhinolaryngology & Communicative Sciences; 2) Medical Genetics Laboratories, Molecular and Human Genetics; Baylor College of Medicine, Houston, TX; 3) Otolaryngology-Head & Neck Surgery, Columbia University, New York, NY.

Mutations in *GJB2* cause hearing loss with or without skin disease. DNA sequencing of *GJB2* in a cohort of hearing impaired patients and a multi-ethnic control group is reported. Among 610 cases, 42 DNA sequence variations were identified in the coding region of *GJB2* including 20 previously identified mutations, 8 polymorphisms, 3 unclassified variants (G4D, R127C, V163V), 1 controversial variant (V37I), and 10 novel variants (G12C, N14D, V63A, T86M, 313_326del, L132V, R134W, D159D, 592_600del_ins17, 647_650del). Sixteen sequence variations were identified among cases in the non-coding region of *GJB2* including 1 mutation, 2 polymorphisms, and 13 novel variants. Two dominant syndromic mutations were detected among cases. A diagnosis of *GJB2*-associated deafness was confirmed for 60 cases (9.8%). Heterozygous mutations were found in 38 cases (6%). Molecular diagnosis was complicated in 30 cases (5%) by the identification of unclassified, novel or controversial sequence variations. In addition, 294 control subjects from 4 ethnic groups were sequenced for *GJB2*. Thirteen sequence variations in the coding region of *GJB2* were identified among controls including 2 mutations, 6 polymorphisms, 2 unclassified variants (G4D, T123N), 1 controversial variant (V37I), and 2 novel variants (R127L, V207L). Nine sequence variations were identified among controls in the non-coding regions adjacent to *GJB2* exon 2. No control was homozygous for a mutation in *GJB2*. Of particular interest among controls were the variable carrier rates and stratification of alleles among ethnic groups, and the complexity of genotypes among Asian controls, 47% of whom carried 2 to 4 DNA sequence variations in the coding region of *GJB2*. The V37I allele occurred among Asian controls at a frequency of 0.076. These data provide new information about carrier rates for *GJB2*-based hearing loss in various ethnic groups and suggest that the V37I variant is a polymorphism.

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Occipital Horn Syndrome in Brothers with a Missense Mutation in the ATP-binding domain of ATP7A. *J. Tang*[1], *S. Robertson*[2], *S.G. Kaler*[1]. 1) Unit on Pediatric Genetics, Laboratory of Clinical Genomics, NICHD/NIH, Bethesda, MD; 2) Department of Paediatrics and Child Health; Dunedin School of Medicine. University of Otago Dunedin NEW ZEALAND.

The clinical spectrum of Menkes disease includes a range of neurological severity, from the classical form that features seizures, severe developmental delay and death by 3 years of age, to occipital horn syndrome (OHS, X-linked cutis laxa or Ehlers-Danlos type IX). Occipital exostoses provide a distinctive radiographic feature in this latter condition, and slightly subnormal intelligence or signs of autonomic dysfunction (e.g., frequent syncope) are the only apparent neurologic abnormalities. While many mutations resulting in classical Menkes disease have been described, the literature contains fewer than ten reports of phenotype-genotype correlations for OHS. These all reflect milder molecular abnormalities, especially involving "leaky" splice junction mutations that impair but do not eliminate proper mRNA splicing. Only two missense mutations (G892D, A1325V) have previously been reported in this phenotype. Here, we report two brothers aged 5 and 14 years with somewhat distinct clinical phenotypes which are each consistent with occipital horn syndrome. In contrast to the two other missense defects producing OHS phenotypes, which do not involve important functional domains of ATP7A, the molecular defect in these brothers (N1304S) lies within the ATP-binding domain of the molecule. While functional analyses are necessary to formally demonstrate the point, the patients' mild phenotypes indicate that the N1304S substitution does not completely disrupt ATP-binding and the consequent ability to transport copper. This finding expands our knowledge of the structure-function relationships for ATP7A and genotype-phenotype correlations in OHS.

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C-reactive protein and homocysteine levels in Prader-Willi syndrome. *N. Kibiryeva, D.C. Bittel, U. Garg, M.G. Butler.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, feeding difficulties, hypogonadism, small hands and feet, mental deficiency, behavioral problems, hyperphagia leading to obesity in early childhood and a paternal 15q11-q13 chromosome deletion in the majority of subjects. Whether the obesity associated with PWS contributes to an increased risk for diabetes and cardiovascular disease is unclear. To date there have been no studies confirming an associated risk of cardiovascular disease relating to obesity in PWS, nor of circulating biomarkers known to be predictive of cardiovascular disease. Therefore, we measured the levels of C-reactive protein (CRP) and homocysteine (HCY), both biomarkers for cardiovascular disease, using commercially available immunoassays from Diagnostic Products Corporation (DPC) on a cohort of PWS and comparison subjects. The means and standard deviations for CRP and HCY in eight nonobese comparison subjects were similar to established means and standard deviations reported by DPC from healthy adults. The mean circulating CRP concentration for 29 PWS subjects (14 females, 15 males; mean age 24.6 +/- 11.6 years; mean body mass index (BMI) 35.9 +/- 11.9) was 1.03 +/- 0.88 mg/dL and the mean HCY value was 5.76 +/- 3.35 umol/L. The mean CRP concentration for 23 nonsyndromic obese subjects (17 females, 6 males; mean age 32.7 +/- 12.5 years; mean BMI 34.6 +/- 5.9) was 0.88 +/- 1.07 mg/dL and the mean HCY value was 6.52 +/- 1.86 umol/L. The reported mean value for CRP was 0.26 +/- 0.30 mg/dL from 100 healthy adults and the mean HCY value was 7.82 +/- 1.8 umol/L from the DPC data. The CRP and HCY values were not significantly different between the PWS and obese subjects. However, CRP levels were significantly higher in both PWS and obese subjects relative to the reported DPC data ($p < 0.001$ for both) but HCY levels were lower in the PWS and obese subjects compared to the DPC data ($p < 0.001$ and 0.01, respectively). Moderately increased levels of CRP (between 0.3 and 1.0 mg/dL) are reportedly associated with cardiovascular disease suggesting both the PWS and obese groups are at an increased risk.

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A new camptodactyly syndrome with lens subluxation and vascular features in two Mexican siblings. *J. Garcia-Ortiz[1], D. Garcia-Cruz[4], M.O. Garcia-Cruz[3], Z. Nazara[3], J. Sanchez-Corona[2].* 1) Division of Genetics, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) Division of Molecular Medicine, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 3) UMAE, CMNO-IMSS, Guadalajara, Jalisco, Mexico; 4) CUCS-University of Guadlajara, Guadalajara, Jalisco, Mexico.

Camptodactyly, a permanent non-traumatic flexion contracture of one or more proximal interphalangeal (PIP) joints of the fingers, is an isolated or syndromic feature; more than 60 distinct camptodactyly entities have been described. Here we report two brothers of 31 and 29 year-old that were seen at the Clinical Genetics service due to lens subluxation, asymmetric face, prominent supraciliar arches, deep nasofrontal angle, low nasal root protrusion, broad and beaky nose, long philtrum, downturned corners of the mouth, retrognathia, ears with hypoplastic anthelix and scapha and a groove in the lobe, wide neck, shoulders with promient trapezium; upper extremities with camptodactyly of fingers 3-5 in left hand, tapered fingers 3 and 4 with thickening of the volar surface, cubital deviation of fingers 3-5, thenar and hypothenar hypoplasia, limited abduction of the thumb; right hand showed similar features but more severe camptodactyly and prominent metacarpophalangeal joint of the thumb were observed; lower extremities with increased volume of the right ankle and presence of an old hypopigmentated scar, bilateral hallux valgus. Ophthalmological evaluation revealed anterior segment with nasal superior subluxation of lens in both eyes and central corneal thickening and a small central opacity in the right eye. Angiologic evaluation revealed varicosities in the lower limbs. Laboratory examination including karyotype, tests for metabolic defects gave normal or negative results. Differential diagnosis included Marfan, Ehlers-Danlos, Weill-Marchesani, Freeman-Sheldon, Tel-Hashomer camptodactyly and camptodactyly-arthritis-coxa vara-pericarditis syndromes. The striking skeletal, ocular and vascular features found in these two siblings are distinguishable from previously reported camptodactyly syndromes that we propose would be considered a new clinical entity.

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Foramen Magnum and Cervical Vertebral Abnormalities in CDX2. *D. Myles Reid[1,2], J. Kingdom[1], A. Toi[1], D. Chitayat[2,3].* 1) Dep. of Obstetrics and Gynecology; 2) The Prenatal Diagnosis and Medical Genetics Program; Mount Sinai Hospital; 3) Div. of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Chondrodysplasia punctata, Conradi-Hunermann Type (CDPX2) is a rare, X-linked dominant condition characterized by asymmetric shortening of the long bones, cataracts; facial flattening with hypoplastic nasal bone in infancy, scoliosis, alopecia, joint contractures, and skin lesions. The condition is associated with mutations in the EBP gene on Xp11. We report a female neonate with CDPX2 who was born at 39 weeks gestation.Parents are healthy and non-consanguineous. Fetal U/S performed at 19 and 20 weeks gestation demonstrated normal growth and segmentation abnormality in the thoracic spine. Fetal MRI at 23 weeks gestation showed a mild alignment abnormality of the spine with kyphosis centered at the thoracolumbar spine. A repeat fetal U/S at 35 weeks gestation showed asymmetrical shortening of all the long bones. There were mild epiphyseal ossifications and a segmentation error or additional ossifications centers along the spine with mild kyphosis of the thoracolumbar junction.At birth the face was asymmetrical with the right side being more prominent than the left. The upper limbs were mildly asymmetric and thyere were streaks of hypopigmentation on the face, trunk and limbs. Cataract was present in the right eye. Stippling was detected on skeletal survey and there were limitations in finger movements. CT scan showed a tight craniocervical junction with what appeared to be compression by the cartilaginous arch of C1. MRI detected a narrowing of foramen magnum and narrow AP dimension of the cervical canal at C1 level consistent with spinal stenosis.The canal was narrowed by approximately 50%. The incidence of a narrow foramen magnum and cervicomedullary compression in cases with CDPX2 need to be further investigated since it can result in neurological problems, leading to disabilities in locomotion, communication, learning as well as sudden death.Early clinical and MRI evaluations may be necessary since early recognition, and immediate decompression can be performed safely to avoid serious complications.

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Association between ADRA2A genotypes and Bone Density in Female Schizophrenic Patients with Exposure to Atypical Antipsychotics. *T. Lan[1,2,3,4], M. Lee[6], H. Chiu[2,5], T. Hu[2], F. Chu[7], H. Sun[8], C. Shu[9].* 1) Inst Genetics, Natl Yang-Ming Univ, Taipei, Taiwan; 2) Psychiatry, Yu-Li Hospital, DOH, Hualien, Taiwan; 3) Division of Mental Health and Substance Abuse Research, NHRI, Taipei, Taiwan; 4) Inst Human Genetics, Tzu-Chi Univ, Hualien, Taiwan; 5) Psychiatry, Natl Yang-Ming Univ, Taipei, Taiwan; 6) Department of Psychiatry, Yi-Lan Hospital, DOH, Yi-Lan, Taiwan; 7) Clinical Biochemical Laboratory, Tao-Yuan Hospital, DOH, Tao-Yuan, Taiwan; 8) Department of Psychiatry, Tao-Yuan Psychiatric Center, DOH, Tao-Yuan, Taiwan; 9) Bureau of Medical Affair, DOH, Taichung, Taiwan.

A lower bone density is suspected prevalent among female schizophrenia patients. This study is to explore the association of bone density in female schizophrenic patients and ADRA2A gene. This is a multi-center, investigator-initiated, naturalistic study project. Here we enrolled 90 female inpatients meeting DSM-IV criteria for schizophrenia or schizoaffective disorder from two psychiatric hospitals in Taiwan. Bone density indicated in BUA was measured for all subjects by using QUS-2 over calcaneal bone. Descriptive data of age, sex, height, body weight, medical or surgical past history, activity level, smoking and the antipsychotics used in the past two years were collected. Four candidate genes including ADRA1A, ADRA2A, ADRB3, HTR2A have been genotyped by PCR or DHPLC. After controlling for gender, age, smoking status, education year, BMI, daily energy intake, and the other 3 genotypes, the absolute regression coefficient of BUA for an individual female with one more allele C(1291) is -5.922.68 (p-value =0.030). After further stratified by their previous treatment of antipsychotics, this association was only held in those taking atypical antipsychotics (p-value =0.020). It is suggested that the SNP C1291G in ADRA2A gene is significantly associated with bone density in our female schizophrenic patients.

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Clinical follow up of five patients with Rothmund Thomson: Importance of osteosarcoma. *C.R.L. Silva, D.R. Bertola, L.M.J. Albano, V. Odone, C.A. Kim.* Pediatrics, Instituto da Criança, São Paulo, Brazil.

Rothmund Thomson Syndrome (RTS) is a genodermatosis characterized by poikiloderma in sun exposed areas, premature aging, sparse hair, thin browns and eyelashes, micro or anodontia, juvenile cataracts and skeletal abnormalities. A helicase family gene (RECQL4), whose mutations have being related to RTS phenotype, was described in a group of patients. RTS is divided in: Type I without osteosarcoma; and Type II with increased risk to develop osteosarcoma and mutations in RECQL4 gene. We report on the natural history of 5 patients followed between 1995-2005, with RTS clinical diagnosis, according to Wang (2001) criteria. All cases were sporadics and from healthy and nonconsanguineous parents. The clinical diagnosis was made between 1y11mo and 29y8mo (mean=9y8mo). They were followed for about 4.8 years and their current ages range from 3y3mo to 30y7mo. The main findings were: poikiloderma in face, thorax and extremities (5/5 - 100%); thin eyelashes (5/5 - 100%); short stature (4/5 - 80%); skeletal abnormalities (4/5 - 80%); microcephaly (2/5 - 40%). None of them developed either cataract or skin cancer. Only one patient presented at 11 years of age osteosarcoma in the left tibia and received chemotherapy for a year without satisfactory response, requiring limb amputation. Poikiloderma injury in precocious ages is the main criteria for the clinical suspicion of RTS. Osteosarcoma occurs in the second decade of life, in up to 30% of the affected patients, and skin cancer occurs in 5% at any age, although earlier than in general population. Thus, a clinical and laboratorial follow up of these patients is extremely important to prompt detection of skin and bone cancers. Molecular study has been shown helpful to identify patients with risk of osteosarcoma.

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Sprengel's deformity associated with Klippel Feil Syndrome in a Mexican patient. A report case at the Hospital Para el Niño Poblano. *C. Gil[1,7], J.M. Aparicio[2,7], M. Barrientos-Pérez[3], E. Landini-Maldonado[4], W.B. San Martín[5], F. Guzman-Serrano[1], K. Sanchez-Ortega[1], M. Gil-Barbosa[6], L.A. Gonzalez-Salazar[7], R. Tenorio-Sánchez[7], C.F. Salinas[8].*
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INTRODUCTION. Eulenberg first described the Sprengel deformity in 1863. Sprengel then described 4 patients of upward displacement of the scapula in 1891. In 1912, Maurice Klippel and Andre Feil were independently the first to describe Klippel-Feil syndrome. They described patients who had a short neck, increased range of motion in the cervical. PROBLEM. Sprengel deformity is a complex anomaly associated with malposition and dysplasia of the scapula with muscle hypoplasia or atrophy, which causes disfigurement and limitation of shoulder movement. Whether Klippel Feil is caracterized by cervical fusion with movement limitations. FRECUENCY. Sprengel deformity is the most common congenital malformation of the shoulder with a male-to-female ratio of 3:1. Meanwhile, the incidence of Klippel-Feil syndrome is unknown. CLINICAL CASE. A Mexican male with unilateral elevation of right scapula short neck; a low posterior hair line; limitation of movement with congenital cervical sinostosis, cleft palate and a dismorfic phenotipe was estudied. CONCLUSIONS. The hallmarks of Sprengel are shoulder asymmetry and restriction of shoulder abduction. The scapula is hypoplasic. However cervical fusion and short neck is frecuently observed in Klippel Feil syndrome. An unusual presentation of Klippel-Feil syndrome was observed in this study, a patient two-year-old boy with multiple associated congenital anomalies that included short neck; a low posterior hair line; limitation of movement with congenital fusion cervical vertebrae (triad seen in 50% of the patients with KF. The patiente also has unilateral elevation of right scapula (seen in 20-25% of Sprengel).

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A New Case of Fibula Agenesis and Oligodactylia Asocciated to Hypospadias and Cryptorchid Testes. *P.L. Paez*[1], *J.C. Prieto*[1,2]. 1) Instituto de Genetica Humana, Bogota, Colombia; 2) Hospital la Victoria, Genetica, Bogota Colombia.

We present a new case of a three year-old boy born in Chaparral (Tolima, Colombia) of non consanguineous parents presents with oligodactylia of hands and feet, bilateral fibula agenesis, bilateral talus deformity of feet, grade II hypospadias and bilateral cryptorchid testes. As this combination has been unpublished before, we believe that this is a new syndrome. The patient was the products of the mother's fourth unterm 34-week pregnancy and cesarean delivery. Prenatal ultrasonographic study showed inespecific dwarfism. There was evident oligodactylia of hands and feet, bilateral fibula agenesis, bilateral talus deformity of feet, grade II hypospadias and bilateral cryptorchid testes at birth. No delayed psychomotor development. No familiar history. R banding karyotype was normal: 46 XY. Roentgenologic findings are: bilateral fibula agenesis, tibial bilateral antecurvatum, delayed bone maduration on carpogram (bone age of two years), right hand: oligodactylia, fifth finger agenesis, thirth methacarpian hypoplasia, fifth finger clinodactylia; left hand: clinodactily and middle phalanx of second finger, bilateral oligodactylia of feet (fourth and fifth fingers agenesis), hypoplasic tarsal bones. Aplasia or hypoplasia of the fibula can occur as an isolated finding or as part of a syndrome. One of the commonest forms is Fibula aplasia/hypoplasia which is associated with short stature, mesomelic shortening, tibial aplasia/hypoplasia, talus or equinovarus deformity, toe hypoplasia and ocasionally fémur hypoplasia. Another condition is Fibula agenesis with oligodactylia and camptomelia which is characterized too for tibial aplasia/hypoplasia, clinodactylia, ectrodactylia, methacarpian agenesis, limb reduction and femur is usually not shortened. All these cases are sporadic and no mendelian inheritance mechanism have been found. No preview literature evidence of association between fibula agenesis, oligodactylia and hypospadias with cryptorchid testes has been reported.

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Identification of a major recombination hotspot in short patients with SHOX deficiency. *G. Rappold[1], N. Sabherwal[1], K. Jantz[1], R. Roeth[1], N. Muncke[1], W.F. Blum[2], G.B. Cutler[3], K. Schneider[1].* 1) Inst Human Genetics, Univ Heidelberg, Heidelberg, Germany; 2) Lilly Research Laboratories, Eli Lilly and Company, Bad Homburg and University Childrens' Hospital, Giessen, Germany; 3) Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA.

Aside from the influence exerted by environmental and internal factors, growth is orchestrated by a large number of different genes. One of them, SHOX, is believed to play a major role since defects in this homeobox-containing short stature gene on the sex chromosomes lead to syndromal (Léri-Weill, Langer and Turner syndrome) or idiopathic short stature. We have analysed 118 independent patients with Léri-Weill dyschondrosteosis and 1,500 patients with idiopathic short stature for deletions encompassing SHOX. Deletions were detected in 34% of the patients with Léri-Weill dyschondrosteosis and 2% of the patients with idiopathic short stature. For 27 patients with Léri-Weill dyschondrosteosis and 6 with idiopathic short stature, detailed deletion mapping was carried out. Analysis was performed by polymerase chain reaction (PCR) using pseudoautosomal polymorphic markers and fluorescence in situ hybridisation (FISH) using cosmid clones. Here we show that the identified deletions vary in size, yet the vast majority (73%) of patients tested share a distinct proximal deletion breakpoint. We propose that the tandem repeat sequences present within this proximal deletion breakpoint hotspot region predispose to recurrent breaks. Based on the prevalence of this deletion breakpoint in idiopathic short stature (1%), SHOX deletions are estimated to occur in roughly 1:2,000 in the total population. This implies that the recurrent deletion breakpoint identified in this study may represent one the most frequent known deletion breakpoint leading to disease. Together, mutations in the SHOX gene are seen in 3% of patients with idiopathic short stature and 60% of patients with Léri-Weill dyschondrosteosis.

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Growth charts for weight, height and other anthropometric measurements in children with skeletal dysplasias.

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Accurate assessment of growth parameters in skeletal dysplasias patients is problematic with current growth curves. Most were constructed from a small number of patients with a paucity of longitudinal data. Furthermore, data were compiled from multiple clinical settings using potentially non-standardized observational methods, and the curves were derived from very basic parametric analysis. Of clinical significance, weight-for-age norms are currently unavailable, despite significant negative orthopaedic, neurologic and general health sequelae caused by unrecognized and untreated obesity in this patient population. We have collected extensive, longitudinal anthropometric data from medical records of patients with a variety of skeletal dysplasias. The majority of the data were from patients with achondroplasia (n=334), with >2000 datapoints for height, weight and head circumference. Other skeletal dysplasias considered include hypochondroplasia, spondyloepiphyseal dysplasia congenita, diastrophic dysplasia, and Morquio syndrome. Patients were born between 1931 and 2004, and all anthropometric measurements were obtained by a single observer (Scott). Gestational age and birth weight, length and head circumference were also analyzed for each skeletal dysplasia type. Percentiles (5, 25, 50, 75, and 95th) were estimated across the age continuum for each growth parameter using a moving 6 month window. Percentiles were then smoothed using a quadratic, penalized smoother. This improves upon previous approaches for generating growth curves by taking advantage of the longitudinal nature of the data, thereby improving the precision of the percentile estimates. New growth charts constructed from these data conform to the current CDC curve structure for average stature individuals, with one chart for each anthropometric parameter for ages 0-36 months and another for 2-20 years.

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Absence of MMP2 Mutations in Idiopathic Multicentric Osteolysis with Nephropathy in 2 American Boys. *D. Wenkert[1], S. Mumm[2], S.M. Wiegand[2], W.H. McAlister[3], M.P. Whyte[1,2].* 1) Research Center, Shriners Hospt, St Louis, MO; 2) Dept Med &; 3) Radiol, Wash U Sch Med, St. Louis, MO.

In 2001, Saudi Arabians with multicentric osteolysis and arthritis syndrome were found to have mutations of the gene encoding matrix metalloproteinase 2 (MMP2) (Nat Gen, 2001, 28:261). They suffered crippling arthritis, subcutaneous nodules, and osteoporosis with carpal-tarsal destruction called Nodulosis-Arthropathy-Osteolysis Syndrome (NAO) (OMIM #605156).

We report 2 unrelated Caucasian boys with idiopathic multicentric osteolysis (IMO) and nephropathy without MMP2 mutation. Elsewhere, each had been diagnosed with Juvenile Rheumatoid Arthritis (JRA) and had received NSAIDs, prednisone, gold, and methotrexate with minimal effect. Patient 1 crawled on knees and elbows at 1 yr of age. X-rays showed "delayed bone age". Flares of pain and limited motion of feet, wrists, elbows, and perhaps knees persisted despite JRA treatment. After IMO was diagnosed, pamidronate was administered for 1 yr. After referral at age 8, mild proteinuria developed over the next 1/2 yr. Osteolysis was treated with oral alendronate. Patient 2, referred at age 7, presented at age 2 1/2 with a limp. Originally evaluated for muscle disease, he was diagnosed with JRA at age 3. He had pain and swelling of wrists and ankles. IMO was diagnosed at age 5 following elbow, knee, and shoulder contractures and bony erosions. Proteinuria and progressive renal disease led to kidney transplantation at age 17. All 13 exons and adjacent mRNA splice sites of the MMP2 gene were amplified by PCR, using genomic DNA. Amplicons were sequenced in both directions, using the same PCR primers. Sequencing chromatograms were inspected and sequences aligned using VectorNTI AlignX software. No MMP2 mutations were detected.

Originally diagnosed as JRA, early clinical clues to IMO included aggressive symmetric involvement of wrists and ankles prior to the involvement of other joints, x-ray reading of delayed bone age, and negative serologies. Mutational analysis supports the clinical impression that IMO represents a disease distinct from NAO.

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Clinical study of five patients with Trichorhinophalangeal syndrome. *L.M.J. Albano, T.B. Brasil, M.B. Moreira, C.R.L. Silva, D. Bertola, C.A. Kim.* Genetics Unit, Inst Da Crianca, Hosp das Clinicas, Sao Paulo, Brazil.

Trichorhinophalangeal syndrome (TRPS) is an autosomal dominant disorder, characterized by sparse scalp hair, bulbous nose tip, long flat philtrum and protruding ears and skeletal abnormalities, such as cone-shaped epiphyses at the phalanges, hip malformations, and short stature. There are 3 subtypes. Type I and II share the above characteristics, but type II differs from type I because of mental retardation and exostosis. In type III we observe normal development, absence of exostosis, but a severe brachydactyly. The responsible genes are located at the 8q.24. We studied 5 TRPS patients from healthy and nonconsanguineous parents. All cases (100%) had sparse hair, bulbous nose, prominent ears and high nasal bridge. In four of them (80%) we found: thin upper lip, elongated facies, displastic ears and ulnar deviation of the fingers. In three patients (60%), sparce eyelashes, long philtrum and recurrent infections were present. Only two of them (40%) had fragile nails and development delay. Abnormalities in the radiographic study were presented in all of them, especially cone-shaped epiphysis (80%). G-banded karyotype showed a deletion in the 8q.24.1 region in only one case presenting typical findings of TRFS II plus cleft lip and palate and preaxial polydactyly in the right hand. Preaxial polydactyly as well as cleft palate was previously described in association with TRFS II. Once this subtype is considered a contiguous gene syndrome with deletion of TRPS1 and EXT1 genes, the presence of these anomalies in our TRPS II patient reinforces the fact that these associations could be not fortuitous and raises the possibility of the presence of other gene(s) responsible for them located in this region.

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Cone-rod dystrophy and spondylometaphyseal dysplasia. *B. Hall.* Greenwood Genetic Center Greenwood, SC.

In 2004 Walters et al. (Am J Med Genet 129A:265) reported 8 cases of spondylometaphyseal dysplasia (SMD) who also had cone-rod dystrophy or were suspected of having cone-rod dystrophy. Three of their cases were mixed sex siblings and one of the isolated cases was the product of first cousin mating. They noted the rarity of occular and non-bony abnormalities in SMD. The radiological features of platyspondyly, characteristic vertebral body shape, irregular cupped metaphyses, bowed long bones, and rhizomelia were felt to be different from other well-established disorders with SMD. I present MZ Black twins born to consanguineous parents one had negative FGFR3 testing, with "probable cone-rod" dystrophy and similar SMD as the cases reported by Walters et al.

The female twins were born at 34 weeks gestation to a 32 year old G2P3 mother who was a distant cousin to the 42 year old father. Twin 1 was 1497g and 38cm and twin 2 was 1391g and 36.8cm, respectively. Genetic evaluation at 4 months revealed both twins to be short with flat facies and bowed, disproportionately short limbs. Twin 2 had a disproportionately large head. At 10 months, achondroplasia was suspected, but both girls were noted to have nystagmus and skeletal x-rays showed ovid vertebrae and more irregular metaphyses than expected for achondroplasia. By 2 years of age non-specific retinal abnormalities were noted and cone-rod dystrophy was suspected. Twin 1 requires glasses. Both girls are developmentally normal.

The twin girls reported herein further support Walters et al contention that there is a specific SMD and cone-rod dystrophy syndrome with probable autosomal recessive inheritance.

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Swallowing dysfunction in Stuve-Wiedemann syndrome. *J.R. Corona-Rivera[1,2], P. Coello[2], E. López[2], H. Silva[2], J. Hernández[2], A.M. de León[2].* 1) Instituto de Genética Humana Dr. Enrique Corona, CUCS, Universidad de Guadalajara; 2) División de Pediatría, Hospital Civil de Guadalajara Dr. Juan I. Menchaca, Guadalajara, Jal., México.

Stuve-Wiedemann syndrome (SWS) is an autosomal recessive skeletal dysplasia mostly considered to have an early lethality. We describe a female infant with SWS who suffered multiple life-threatening events. Cause of death or morbidity in SWS is discussed. She was the second child of healthy second cousin Mexican-gypsy parents. After birth, respiratory distress and severe episodes of hyperthermia and apnea were observed. Evaluation at 8 months of age showed weight 4200g (-5.7 SD), length 56 cm (-5.8 SD), short wide nose, hypolacrimation, lack of corneal reflexes, smooth tongue, bowing of the long bones, camptodactyly, overlapping fingers, hearth murmur, hypotonia and reduced patellar reflexes. On X-ray showed osteoporosis, bowing of the long bones with abnormal trabecular pattern, wide metaphyses and scoliosis. Electromyography showed absence of sympathetic skin response. She showed absence of reaction after intradermic injection of histamine and myosis after instillation of methacholine. Echocardiography revealed pulmonary hypertension. On examination of barium swallow showed aspiration of oropharyngeal secretions. Esophagic aperistalsis was found on manometry. Results of cranial CT scan, abdominal ultrasound, metabolic screening test and karyotype were normal. At two months of age, despite nasogastric feeding, she showed repeated pulmonary aspirations and required an esophagectomy and feeding gastrostomy. An ectopic thyroid gland was found at surgery. After these measures, no one event of aspiration pneumonia was experienced. Also, oxygen dependence, temperature instability, reduced sweating and pain sensations, and spontaneous fracture of the radius, were observed on evolution. Since our patient had symptoms of dysautonomia as well as evidence of esophageal dysfunction on manometry and barium swallow, we propose that swallowing dysfunction could be a dysautonomic manifestation of SWS. Early surgical management in infants with SWS could prevent morbidity or inclusively early death.

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Cranioectodermal dysplasia: hepatic manifestations in two unrelated children. *C.H. Gonzalez[1], C.Y Utagawa[2], N. Zannon-Collange[2], M.R. Passos-Bueno[1], F. Sarquis-Jahee[1].* 1) Dept Genetics & Evol Biology, Inst de Biociencias - USP, Sao Paulo, Brazil; 2) Clinical Genetics, Pediatric Neurosurgical Division, Hospital Santa Marcelina, São Paulo, SP, Brazil.

Cranioectodermal dysplasia (CED) also known as Seusenbrenner Syndrome, is an autosomal recessive condition characterized by craniofacial and skeletal anomalies and by ectodermal dysplasia. There are 15 cases described in the literature including 4 pairs of brothers and a set of monozygotic twins. Manifestations of ectodermal dysplasia are variable. All the patients presented dolichocephaly and most of them, rhizomelia. The skull is scaphocephalic with sunostosis of the sagittal suture. Several children have died from chronic renal failure. Heart defect and short thorax were also observed as well as growth deficiency, delayed psychomotor development, microcephaly and abnormal calcium homeostasis. We have studied two unrelated children affected by CED. Patient 1 is a 9-year-old boy, the first child of healthy and non-consanguineous parents. Patient 2 is a 8-year-old girl born from a consanguineous couple. Both patients presented doliocephaly, prominent sagittal suture, frontal bossing, fine, sparse and slow-growing hair, peculiar facies with mongoloid slanting of the palpebral fissures, epicanthic folds, sparse eyebrow and long philtrum. The thorax was narrowed and at the hands brachidactyly and partial cutaneous syndactyly were observed. Joint laxity and hyperelasticity of the skin were also seen. In both patients sinostois of the sagittal suture was diagnosed and they were submitted to a neurosurgical procedure. Microdontia was observed during the follow-up. Both children also presented progressive hepatomegaly with ascites since 4 years of age. Patient 1 also had renal manifestations and his diagnosis was parenchimatosal nephropathy. His liver biopsy revealed moderate septal fibrosis. Patient 2 has no renal problem and her liver biopsy was not perfomed. We believe that the hepatic involvement observed in these two cases offer further evidence that the complete phenotype of CED was not described yet.

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A new distal arthrogryposis syndrome characterized by plantar tendon contractures: clinical analysis of a large Utah kindred. *D.A. Stevenson[1], A. Rutherford[2], K. Swoboda[3], M. Bamshad[1,2].* 1) Dept Pediatrics, Univ of UT, SLC, UT; 2) Dept of Human Genetics, Univ of UT, SLC, UT; 3) Dept of Neurology, Univ of UT, SLC, UT.

Distal arthrogryposis (DA) syndromes are caused by mutations in genes (i.e., TNNI2, TNNT3, MYH7, and TPM2) encoding components of the contractile apparatus of fast-twitch myofibers. Accordingly, phenotypic and molecular characterization of DA syndromes is providing novel insights about the mechanism by which defects of contractility cause congenital contractures. We characterized a large three-generation Utah family in which plantar tendon shortening was transmitted among 13 affected individuals in an autosomal dominant pattern. Contractures were not evident at birth but became apparent in early childhood with the onset of walking. Indeed, plantar tendon shortening was commonly manifest as toe-walking, and required surgical palliation (i.e., tendon lengthening) in 8 cases. Contractures of hips, elbows, wrists, and fingers were variable among affected individuals and of modest severity. No affected individuals had clubfeet, hip dislocation, or clenched fists. All affected individuals had normal neurological and ocular exams, and normal stature. Creatine phosphokinase levels, magnetic resonance imaging of the lower leg and ankle, electromyograms and electrocardiograms on selected individuals were also normal. This disorder meets the diagnostic criteria for a DA but is distinct from all other DAs and appears, to our knowledge, to be unique. A genome-wide linkage scan to identify candidate loci is underway.

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Amelogenesis Imperfecta (AI) and Nephrocalcinosis: further delineation of a rare or unrecognized syndrome. *I. Bailleul-Forestier[1,2], M. Molla[2], G. Zanni[3], D. Griffiths[5], B. Llanas[6], Ch. Loirat[7], D. Lacombe[5], A. Berdal[2], A. Verloes[3,4].* 1) Dpt Stomatology, Robert DEBRE Hospital, Paris; 2) INSERM U714; 3) Clinical Genetics Unit, dpet of Genetics, Robert DEBRE Hospital, Paris; 4) INSERM U676; 5) Dpt of Medical Genetics. Pellegrin University Hospital, Bordeaux; 6) Dept of Paediatric Nephrology, Pellegrin University Hospital, Bordeaux; 7) Dept of Paediatric Nephrology, Robert DEBRE Hospital, Paris, France.

AI is a clinically and genetically heterogeneous group of disorders of enamel development. Enamel defects can occur in isolated or syndromic forms. Mutations in genes encoding for enamel matrix proteins (Amelogenin X, Enamelin, Ameloblastin ...) have been identified respectively in XLR and AD families with apparently non-syndromic AI. The AI-Nephrocalcinosis (AIN) or renal-enamel syndrome is an AR condition with only few reported cases in the literature. Renal symptoms include progressive nephrocalcinosis without any apparent abnormality in calcium metabolism that could lead some patients to renal failure in the adulthood. At present no mutation have been identified in this syndrome. Albumin, osteopontin, calbindine-D28k and calcium sensing receptor could be proposed proteins. These four proteins are involved in calcium metabolism, in tooth and kidney physiopathology, and are expressed in both teeth and kidney cells. We report two consanguineous families and three sporadic cases of AIN. Generalized enamel hypoplasia of both primary and permanent dentition was present in all affected patients. In two families delayed or absent molar eruption and microdontia were noted. None of the patients had impaired renal function. In one sibship, nephrocalcinosis was only present in 1/3 AI patients. As nephrological screening of AI patients is not systematically done in AI, the incidence of this syndrome could be underestimated, and the risk of renal failure overestimated. As early diagnosis may lead to a better renal prognosis, all children with AI should at least have one renal ultrasonography performed to exclude nephrocalcinosis, and this examination should be repeated through infancy, as the natural history and age of onset are not known.

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A new clinical finding in the Richieri-Pereira type of Acrofacial Dysostosis. *L.O. Dewes[1,2], V.R Philipsen[1,2], M.B. Golbert[1,2], R.S. Wachholz[1,2], C. Deutschendorf[1,2], R. Giugliani[1,2], J.C.L. Leite[1,2].* 1) Medical Genetics Service, Hosp Clin P Alegre, Porto Alegre , RS, Brazil; 2) Faculdade de Medicina,Universidade Federal do Rio Grande do Sul,Porto Alegre, RS.

We present a female newborn, from a 37-week pregnancy, who is the first child of a consanguineous couple without previous family history of congenital malformations. The mother was 24 years old and had na uneventful pregnancy. The patient was born through a cesarian section, weighting 2340 g, with a length of 44 cm and a head circumference of 32 cm. Apgar score was 8 on the first minute. Mother reported the use of folic acid during the first trimester. The physical examination showed severe micrognatia, palpable mobile collarbone and a median mandibular cleft. The radiological study showed mentus agenesia, congenital pseudoarthrosis of the right collarbone, deformities in the distal extremity of the left humerus, acetabular dysplasia and bilateral hip sub-luxation, bilateral clubfoot, hypoplasia pf the first metacarpus and fusion of the fourth and fifth metacarpi, bilaterally. The Richieri-Pereira form of Acrofacial Dysostosis has 16 known and only 9 of them published until 1999, 8 had been born in Brazil and one in France. All the brazilian cases had Portuguese ancestry, and a founding effect is suggested by the authors. It is characterized by short stature, Pierre-Robin sequence, congenital clubfoot, pre- and post-axial anomalies in hands and mandibular cleft. Some children may present malformations of the larynx. Its an autossomal recessive condition, and the mental development of the affected patients is within normal limits. In the present case, the clavicular hypoplasia raises the suspicion of an association with cleidocranial dysplasia, an autosomal dominant condition characterized by clavicular hyplopasia or agenesia, open fontanels, supranumerary teeth, short stature, and other changes in skeletal development of the affected individual. Cleidocranial dysplasia is the result of a mutation involving the CBA1 transcription factor in cromossome. The clavicular hypoplasia may be a new, yet to be described finding in the syndrome. DNA samples were sent for analysis.

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Expanding the phenotypic spectrum of cerebro-costo-mandibular syndrome. *S. Purandare[1], N. UNANUE[3], K.L. Ciprero[1], L. Medne[1], M. Guttenberg[2], E.H. Zackai[1].* 1) Division of Human Genetics and; 2) Department of Pathology and; 3) Clinical Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Cerebro-costo-mandibular syndrome (CCM) is characterized by posterior rib gap defects, and Pierre Robin sequence. Growth retardation and development delay are common. The mortality is over 50% in the first year of life due to hypoxia. The inheritance pattern and the expressivity are variable. We report a patient whose findings of micrognathia, cleft palate and posterior rib gaps were consistent with CCM syndrome. The patient presented with small low set ears, bifid uvula, small narrow chest, lung hypoplasia, abnormal clavicles, absent ossification of the sternum, 11 ribs pairs, right ventricle dilatation, small left subclavian artery and bilateral inguinal hernia, all findings reported in CCM syndrome. In addition the patient was noted to have clenched hands, short proximal upper and lower extremities, bilateral 5th finger clinodactyly, small nails and full 4-5 toe syndactyly. Since the first description of CCM, over 60 patients (Megier, et al) have been reported and limbs defects (clinodactyly, contractures of the large joints, hypoplasia of radius, ulna and phalanges, cutaneous syndactyly) are unusual. A role for genes affecting chondrogenesis is suggested due to costal, palatal, and limb involvement. The presence of limb defects seen in our patient has not previously been reported and expands the phenotypic spectrum of CCM.

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Lowry-Wood syndrome in two new patients. *P. Blanchet, C. Coubes, J. Puechberty, V. Cacheux, G. Lefort, P. Sarda.*
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Lowry-Wood syndrome (LWS) is a rare disease characterized by multiple epiphyseal dysplasia, microcephaly, mild short stature, mental retardation and congenital nystagmus. This condition is probably inherited as an autosomal recessive trait. Presently, the gene defect of this disorder is not known. To date only 7 patients have been described with the characteristic features of this syndrome. We report two new cases in two different families. The first boy, 10-years-old, is the only infant born of non-consanguineous parents. At birth, he presented intrauterine growth retardation (height: 41cm, weight: 1960g and OFC: 30cm for 36 weeks' gestation). Postnatal short stature (-4.5SD) and microcephaly (-2.8SD) were confirmed, associated with mental retardation and chronic bronchopathy. Multiple epiphyseal dysplasia was present on skeletal X-rays. The second case concerns a first boy, 14-years-old, born after three normal sisters from non-consanguineous parents. At term weight and OFC were normal but height was 46cm. The boy developed psychomotor retardation, microcephaly (-3SD), seizures and short stature (-4SD). Additional findings included bronchial dilatations of left lower lobe which were surgically corrected and precocious puberty. The two boys had no growth hormone deficiency. Hormonal treatment was administered to the first infant but was relatively inefficient (+1SD on 6 years). At the young age of these patients, no congenital nystagmus and no other major visual problems were present. These two new observations confirm the classical phenotype of patients with Lowry-Wood syndrome and suggest that large expressivity is present in this syndrome.

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Opitz C (trigonocephly) syndrome in a boy with reciprocal translocation t(3;18)(q13.13q12.1). *Y. Chinen[1], T. Kaname[2], K. Yanagi[2], K. Naritomi[2].* 1) Dept Pediatrics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 2) Dept Medical Genetics, Univ Ryukyus Sch Medicine, Okinawa, Japan.

Opitz trigonocephaly C syndrome (OTCS) is a multiple congenital anomaly syndrome characterized by trigonocephaly, mental reterdation, typical facial appearance, redundant skin, joint and limb abnormalities and visceral anomalies. Several reports on the patients suggest that there is heterogeneous condition of the OTCS.
We report on a patient with clinical features of OTCS having a de novo translocation. The proband was 5-month-old at first visit with trigonocephaly, high-arched palate, thick and irregular alveolar ridge, hypotelorism, long philtrum, redundant nuchal skin, and hypotonia. His CT-scan on the head shows agenesis of corpus callosum. The clinical features and facial appearance in the patient are consistent with the OTCS. High resolution G-banding analysis revealed that he had a balanced translocation t(3;18)(q13.13q12.1) de novo.
These findings suggest that there is a possibility of a new locus responsible for OTCS on 3q13.13 or 18q12.1.

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Fibrodysplasia Ossificans Progressiva: Middle-Age Onset with Poor Response to Aggressive Immunosuppressive Therapy. *S.J. DiMartino[1], E.F. DiCarlo[1], A.L. Boskey[1], M.P. Whyte[2,3].* 1) Hospt Special Surgery, New York, NY; 2) Wash U Schl Med, St. Louis, MO; 3) Shriners Hospt, St Louis, MO.

Fibrodysplasia ossificans progressiva (FOP) features ectopic bone formation and congenital malformation of great toes. The gene defect is unknown with no established treatment. Early lesions present as warm, erythematous, and tender nodules. Histology shows proliferating fibroblasts with sparse inflammatory cells. Lesions then ossify by an endochondral process. Ossification typically develops before 15 yrs of age (avg 3-5 yrs). A woman with FOP first developed ectopic ossification at age 47 yrs. Symptoms began with progressive pain, redness, warmth, and swelling over a scapula. CT showed asymmetric thickening of muscles and fascial planes, but no ectopic bone. Biopsy revealed proliferating fibroblasts with scattered inflammatory cells. Prednisone, 60 mg/d, was started for suspected inflammatory fasciitis. Symptoms improved, but returned during steroid taper on the posterior thorax, flank, and chest. Despite high-dose corticosteroids, immunosuppressives, and alendronate early on, ectopic bone deposition progressed rapidly. Methylprednisone (24 mg BID), methotrexate (max dose: 25 mg/wk), and alendronate (35 mg/wk) seemed to improve symptoms. Subsequently, however, these worsened, and methotrexate was discontinued due to non-efficacy (total treatment: 14 wks). On high dose steroids and alendronate, new larger lesions developed during trials of azothiaprine (max dose: 125 mg/d, total treatment: 10 wks) and mycophenolate mofitil (max dose: 1 g BID, total time of treatment: 10 wks). CT, 13 mo after FOP onset, showed many regions of ectopic bone in fascial planes of the thoracic, abdominal, and pelvic walls. She had the typical hallux valgus deformity of FOP. Our patient demonstrates that FOP may present well into adulthood when progression may seem inexplicably rapid despite intervention with high-dose steroid, immunosuppressive, and bisphosphonate therapy. It is important to recognize an adult-onset FOP because early diagnosis can help avoid harmful procedures (e.g. biopsy or surgical excision typically exacerbates the disorder) and unnecessary treatment.

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Olliers disease (multiple enchondromatosis) associated with neuroblastoma and congenital cataract. *H. Fryssira[1], M. Moschovi[2], D. Tsoumas[2], F. Tzortzatou-Stathopoulou[2], A. Mavrou[1].* 1) Dept Medical Genetics, Ag Sophia Child Hosp, Athens, Greece; 2) Oncology Unit 1st Department of Pediatrics, Athens University School of Medicine, Athens, Greece.

Olliers disease (multiple enchondromatosis,OMIM 166000) is a non-hereditary mesodermal dysplasia that affects the metaphyseal ends of tubular bones causing multiple enchondromas. We report on a 6.5-year old girl with congenital cataract, neuroblastoma and Olliers disease. Cataract of the right eye was diagnosed at the age of 5 months and laboratory investigation for a metabolic disorder or congenital infection was negative. At the age of 12 months she developed a palpable mass in the right adrenal gland which was surgically removed and biopsy showed that it was a neuroblastoma. She received seven cycles of chemotherapy with OJEC (Vincristine, Etoposide, Cyclophosphamide and Carboplatinum). When the patient was 15 months old a slight length discrepancy and deformity of the rib cage were noted and x-rays of the pelvis and lower limbs showed widespread lytic abnormalities affecting the metaphyses, diaphyses and epiphyses of the long bones, as well as the iliac wings and pubic ramai. Multiple calcific foci were seen within these lesions. There was apparent cortical destruction but overall the appearances were in keeping with multiple enchondromatoses. No phleboliths were visible which made Olliers disease more likely than Maffucci syndrome. An MRI of the brain showed a skull base mass involving the clivus, petrous apices, sella and both cavernous sinuses which displaced the carotid artery. The most likely diagnosis included that of an enchondroma and follow up imaging was recommended in order to distinguish it from a chondrosarcoma. It is reported in the literature that approximately 30% of patients with Olliers disease will develop a secondary malignant bone neoplasm, most likely chondrosarcoma, but the possibility of developing other malignancies is low. We believe that the case described here is the first to be reported in which Olliers disease is associated with neuroblastoma and congenital cataract.

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Mechanic and genetic component of congenital hip dislocation: an association study for analysing the implication of candidate genes. *K. Rouault[1], V. Scotet[1], F. Dubrana[2], B. Fenoll[3], F. Gaucher[4], D. Tanguy[5], C. Yaccoub[6], C. Férec[1].* 1) INSERM U613, Brest, France CHU Morvan, Brest, France University, Brest, France; 2) Department of orthopaedic surgery, CHU La Cavale Blanche, Brest, France; 3) Department of paediatric surgery, CHU Morvan, Brest, France; 4) Department of orthopaedic surgery, Hotel Dieu, Pont L'Abbé, France; 5) Department of physical medecine, Centre de Perharidy, Roscoff, France; 6) Department of orthopaedic surgery, CH Cornouaille, Quimper, France.

Congenital dislocation of the hip (CDH), which is one of the most common skeletal congenital anomalies, is a public health matter because of its high frequency, the severe functional handicap induced if it is not treated early and its natural evolution towards hip osteoarthritis. This disease presents a mechanical component linked to the pregnancy and delivery conditions, but the ethnical predispositions and the familial aggregation observed suggest that it also presents a genetic component. We set up an association study in the area of Finistère (western Brittany, France) where CDH is particularly frequent in order to study the implication of candidate genes. In a first step, we described the epidemiological characteristics of our cohort of patients. To date, 213 CDH patients have been recruited and the cohort is composed of 92.5% of women (n=197 - sex-ratio: 1:12). The pathology was bilateral in 59.0% of cases and when it was unilateral, it affects as often the left than the right hip (50.6 vs 49.4%). Breech presentation was documented in 157 patients and was observed in 12.0% of them, among whom 52.6% were delivered by caesarean. In this cohort, 13.5% of the patients had a high birthweight (4kg) and 5.3% were postmature babies (42 weeks of gestation). CDH affected the first child of the family in 41.1% of cases and three patients were part of multiple pregnancies. On clinical aspects, a postural anomaly was observed in 10.8% of patients. Hyperlaxity was also present in 10.8% of them and femoral antetorsion in 23.4%. This study will report the preliminary results of the first association study made on CDH and will highlight the role of candidate genes in this complex disease.

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Deactivating Germline Mutations in LEMD3 Cause Osteopoikilosis and Buschke-Ollendorff Syndrome, but Not Melorheostosis. *S. Mumm[1], D. Wenkert[2], W.H. McAlister[3], X. Zhang[1], M.P. Whyte[1,2].* 1) Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO; 2) Ctr Metab Bone Dis & Mol Res, Shriners Hospitals, St Louis, MO; 3) Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO.

In 2004, heterozygous loss-of-function mutations in the LEMD3 gene were shown to be a cause of osteopoikilosis (a benign, autosomal dominant, skeletal dysplasia featuring multiple hyperostotic lesions symmetrically throughout the skeleton) and Buschke-Ollendorff syndrome (BOS) (a benign, autosomal dominant disorder combining osteopoikilosis with disseminated connective tissue nevi). In some of these families, unusually large areas of dense bone were called melorheostosis which typically refers to a troublesome sporadic skeletal dysostosis characterized by asymmetrical flowing hyperostosis of the cortex of long bones often with overlying soft tissue abnormalities. Others had proposed that melorheostosis results from a second, post-zygotic, somatic mutation in the putative osteopoikilosis gene. We investigated patients representing 9 families with sclerosing bone disorders where LEMD3 represented a candidate gene abnormality: 1 osteopoikilosis, 2 BOS, 2 melorheostosis, 1 reported with both osteopoikilosis and melorheostosis, and 3 additional patients with bone dysplasias. Genomic DNA, isolated from blood lymphocytes, was amplified by PCR and sequenced for all the coding exons and adjacent splice site regions for the LEMD3 gene. We did not study melorheostosis lesional tissue. A heterozygous nonsense mutation (T1433A, L478X) was found for the osteopoikilosis patient, and a heterozygous nonsense mutation (C1323A, Y441X) and another heterozygous mutation (insertion or deletion in exon 1) were found in the 2 BOS patients. Likewise, a heterozygous nonsense mutation (C1963T, R655X) was detected for the patient with osteopoikilosis and "melorheostosis". However, no LEMD3 mutations were detected for any other patient, including the 2 patients with classic melorheostosis. We conclude that osteopoikilosis and BOS are caused by heterozygous deactivating LEMD3 mutations, however, melorheostosis remains of unknown etiology.

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Hypochondroplasia: FGFR3 gene mutation analysis in Indian pedigrees and possible genetic heterogeneity. *P.S. Gambhir[1], R. Uppala[2], U. Ratnamala[2], R. Meda[2], S. Nath[3], J.V. Solanki[4].* 1) Department of Pediatrics, B. J.Medical College , Pune, Maharashtra, India; 2) Green Cross Blood Bank & Genetics Research centre, Paldi, Ahmedabad,; 3) Arthritis & Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 4) Department of Animal Genetics & Breeding, Veterinany college, Anand, India.

Hypochondroplasia (HCH; OMIM 146000) also called genetic dwarfism is the most common type of skeletal dysplasia in humans. It is characterized by short stature, micromelia, lumbar lordosis, short and broad bones. The skeletal features of hypochondroplasia are comparable to that of achondroplasia but milder, and these two are considered to be allelic. It occurs at a frequency of approximately 1:30,000 births. Mutations in the FGFR3 gene at chromosome 4p16.3 are associated with HCH. A total of 29 loss-of-function mutations or missense mutations have been detected in FGFR3 and over 95% of patients were identified with a common mutation (Gly380Arg) in the gene (Nat Genet. 1995 10:357-9). We analyzed the FGFR3 gene coding regions from three independent large HCH families and 27 sporadic cases of Indian origins and confirmed the presence of G380R mutation only in 9 sporadic patients. No other pathogenic mutations were identified in the FGFR3 gene-coding region in all the remaining patients including three large families with autosomal dominant mode of inheritance. Clinical and radiographic findings were recorded for all the studied individuals. Absence of mutations in FGFR3 and exclusion of large families to the 4p16.3 region by linkage analysis indicates genetic heterogeneity within HCH. Our data also support the previous reports that HCH is clinically and genetically heterogeneous (J. Med. Genet. 33: 749-752, 1996; J. Med. Genet. 32: 492-493, 1995. Systematic genome-wide linkage analyses is in progress to map the elusive locus responsible for HCH in the present Indian families.

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Split-hand/foot malformation with long bone deficiency (OMIM 119100) in a large inbred Arab family with an apparent autosomal dominant inheritance and reduced penetrance: Clinical and Genetic analysis. *M. Naveed[1], M.T. Al-Ali[1], S.K. Murthy[1], S.AH Ali[1], N. Al-Khaja[1], S.K. Nath[2], S. Deutsch[3], A. Bottani[3], S.E. Antonarakis[3], U. Radhakrishna[3].* 1) Center For Arab Genomic Studies (CAGS), PO Box 22252, Dubai, United Arab Emirates; 2) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, USA; 3) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland.

Hereditary developmental anomalies of the upper and/or lower extremities in humans occur in 1:500 to 1:1000 newborns. We have studied a very large six-generation inbred Arab family with multiple severe limb anomalies resembling OMIM 119100, the gene of which is at present unknown. The pedigree consists of 138 individuals including 22 (14 males/8 females) affecteds. Of these, 18 had tibial hemimelia (THM) usually on the right side. The expression of the phenotype was variable and ranged from bilateral to unilateral THM with or without foot anomalies, syndactyly, oligodactyly and split-hand (in one female). The mode of inheritance appears to be autosomal dominant with reduced penetrance. There were also ten consaguineous marriages observed in this pedigree. This also could suggest a recessive mode of inheritance with pseudodominance due to high frequency of the mutant allele. Candidate loci for the described syndrome include the Langer-Giedion syndrome (LGS; 150230) on 8q24.1, Split-hand/Foot malformation 3 (SHSF3; 600095) on 10q24, GLI3 morphopathies (GLI3; 165240) on 7p13, Sonic Hedgehog; (SHH; 600725) on 7q36. In addition bilateral tibial hemimelia and unilateral absence of the ulna was previously observed to co-segregate with deletion of 8q24.1. Two-point linkage analysis and haplotype data did not show the involvement of the above regions in this family. Cytogenetic and FISH analysis for the critical loci on 8q24.1, 10q24, 7p13 and 7q36 also did not show any abnormality. Genomewise linkage analysis is in progress to map the elusive locus and provide a target for positional cloning.

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ARC syndrome in two patients with no mutation in VPS33B. *T. Tohma[1], K. Yanagi[2], I. Nakazato[3], T. Kaname[2], K. Naritomi[2].* 1) Dept Pediatrics, Okinawa Prefectural Naha Hosp, Okinawa, Japan; 2) Dept Med Genet, Univ Ryukyus Sch Med, Nishihara, Okinawa, Japan; 3) Dept Pathology, Okinawa Prefectural Naha Hosp, Okinawa, Japan.

ARC syndrome (OMIM 208085) refers to an association between arthrogryposis, renal tubular dysfunction, and cholestasis. Autosomal recessive inheritance is suggested and the *VPS33B* gene has been reported as responsible for the syndrome. We report on two female patients from one Japanese family with typical phenotypes of ARC syndrome. Patient 1: This girl was third child of healthy, no consanguineous parents. She admitted to our NICU because of constipation and vomiting after birth. She was noted to have hypotonia, arythrogryposis, ichtyosis and renal tubular acidosis. She developed cholestatic jaundice and anemia including thrombocytopenia, and died at the age of 6 month. Patient 2: The female sibling of patient 1 appeared similar symptom but it was milder than her sisters. The liver histology of both patients was characterized by lobular cholestasis and giant cells. Immunostaining with polyclonal antibody to CEA showed diffuse distribution in cytoplasm of the hepatocytes.

Direct sequencing analysis for all the exons (23 exons) in the *VPS33B* gene for both patients defined that they had no mutation in the gene. Besides, semi-quantitative RT-PCR revealed the level of *VPS33B* expression in the patients in B cells and liver were the same as in normal control.

Thus, we conclude that the patients affected with ARC syndrome have no mutation in *VPS33B* and there is heterogeneity of the syndrome.

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Frontometaphyseal dysplasia: Phenotypic diversity and correlations with mutations in *FLNA*. *S. Robertson, Otopalatodigital Syndrome Spectrum Disorders Clinical Collaborative Group.* Dept Pediatrics, Otago Medical Sch, Dunedin, New Zealand.

The otopalatodigital syndrome spectrum disorders (OPDSD) are a group of phenotypically related conditions characterised by a skeletal dysplasia and variable extra-skeletal malformations. Approximately two thirds of individuals with an OPDSD diagnosis have an identifiable mutation within the X-linked gene *FLNA*, although this proportion differs depending on the diagnosis. Frontometaphyseal dysplasia (FMD), a condition belonging to the OPDSD group, is characterised by a skeletal dysplasia and respiratory tract, cardiac and genitourinary malformations. In contrast to other OPDSDs, mutations underlying this diagnosis are dispersed over more than one mutation hotspot within *FLNA*. We describe the molecular and clinical analysis of 20 individuals with frontometaphyseal dysplasia. Mutations underlying this phenotype were found in 10/22 individuals analysed and were observed to cluster in four hotspots within *FLNA*. Mutations were predicted to lead to amino acid substitutions within the actin binding domain, filamins repeats 9-10, 14-16 and 22-23. Males with typical phenotypes were identified with mutations in the three most 5' hotspots whereas only females have been observed with mutations predicting substitutions near the C-terminus of the protein. In two families with mutations within exon 22 (in addition to one previously reported in the literature with a mutation in exon 29), a severe male perinatal-lethal phenotype was noted. A skeletal dysplasia, cardiac anomalies and ureteric and urethral stenosis were the cardinal components of this phenotype. Markedly skewed X-inactivation patterns were noted in all females with an identifiable *FLNA* mutation, whereas a substantial proportion of females with no such mutation identifiable, including one familial instance, did not demonstrate such skewing. We therefore propose that FMD may exhibit locus heterogeneity.

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An Unusual Presentation of Central Ray Deficiency in Manitoba Patients-A Dilemma for Classification. *A.M. Elliott[1], M.H. Reed[1,2,3,4], J.A. Evans[1,3,4].* 1) Department of Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, MB, Canada; 2) Department of Radiology; 3) Department of Pediatrics and Child Health; 4) Children's Hospital, Winnipeg, Manitoba, Canada.

Objective: We performed a clinical epidemiologic study of central ray deficiency (Split Hand Foot Malformation/SHFM, cleft hand/foot) of the Manitoba population. This study was conducted i) to determine the birth rate of SHFM and ii) to perform a classification of patients who met the inclusion criteria.

Methods: One hundred and sixty-four patient files were reviewed. The majority of patients were provided by the Section of Genetics and Metabolism of the Childrens Hospital.

Results and Discussion: In total, 43 patients met the inclusion criteria, resulting in a birth rate of 1 in 19 784. There were 26 females and 17 males. Twenty-nine patients had typical SHFM and 3 had atypical SHFM. There were 11 remaining patients who did not fit neatly into either group and comprised a distinct group, difficult to classify. Within this cohort, we identified 7 patients who had SHFM with extensive cutaneous syndactyly. Various classifications have been proposed for SHFM. Typical versus atypical SHFM was originally differentiated by Lange (1937). Atypical SHFM is usually sporadic and is commonly represented by a deficiency of the three central rays with a web or small nubbins in place of the deficient digits. Typical SHFM usually shows a central V-shaped cleft, more than one limb may be affected and there may be a positive family history. The 7 unclassified Manitoba patients had monomelic involvement, and in all cases it involved an upper limb (2 showed right- and 5 showed left-sided involvement). These 7 patients comprised 16% of our total affected population and represent an intriguing subpopulation. Their phenotype is not representative of either typical or atypical SHFM and has not been well recognized in the literature. We discuss the difficulties with classification and genetic counselling of SHFM patients and emphasize the importance of detailed clinical and radiographic evaluations of these patients and family members.

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Expansion of the phenotype of X-linked hypophosphatemic rickets. *A.L. Shanske, J. Neil, M. Markowitz.* Ctr Craniofacial Disorders, Children's Hosp Montefiore, Albert Einstein College of Medicine, Bronx, NY.

X-linked dominant hypophosphatemic rickets (XLH)is the most common form of hereditary rickets.Children exhibit growth retardation,deformities of the lower extremities and radiographic evidence of rickets.In 1995, mutations in the *PHEX* gene on Xp22.2-p22.1 were found to cause XLH.We recently examined a mother and son with XLH with the Chiari malformation, syringomyelia, symmetric multiple craniosynostosis, ricketic radiographic changes and unique bony changes suggestive of a skeletal dysplasia.MG was the 3500 gm product of a term uneventful pregnancy born to unrelated West Indian parents.His mother, maternal grandmother, maternal half-aunt and her 2 sons all have XLH.An MRI at 5 months revealed hydrocephalus and a Chiari I malformation.A diagnsosis of XLH was made at 2 years and he was begun on a regimen of vitamin D and phosphate.His physical examination at 8 years revealed a short stigmatized delayed youngster whose height was 103.3 cm(<5%), weight 21.7 kg (5%), and HC was 52.5 cm (50%).He had turribrachycephaly, frontal bossing,malar flattening and exophthalmos.He had anterior bowing of the distal radii,flexion contractures of the elbows,bowing of the femora and tibia,notching of the inner maleolus,and a waddling gait.He had normal calcium and PTH levels,elevated alkaline phosphatase and depressed phosphate.A 3D CAT scan showed synostosis of all sutures and significant signs of increased intracranial pressure.A renal ultrasound revealed nephrocalcinosis.A skeletal survey showed in addition to ricketic changes and osteomalacia,diffuse metaphyseal fraying and cupping and widening of the physis,generalized platyspondyly and mild endplate sclerosis,pars interarticularis fractures and L4-L5 retrolisthesis.A survey of the literature indicates an incidence of craniosynostosis of 13% and of Chiari malformation of 44% in patients with XLH.Our patients have additional non-rachitic skeletal changes not previously reported.We plan to perform *PHEX* mutation analysis and suspect that although more than 140 mutations in the *PHEX* gene have been identified, a new allele may be found in this family.Alternatively,XLH in this family may be caused by a novel X-linked gene.

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The Influence OF Speech And Family History on Mood Symptomatology and Autism Spectrum Disorders. *A.V. Hall[1], R.K. Abramson[1], S.A. Ravan[1], H. Cope[2], J. Gilbert[2], M.L. Cuccaro[2], H.H. Wright[1], M. Pericak-Vance[2].* 1) Dept Neuropsychiatry, Univ South Carolina Sch Med, Columbia, SC; 2) Duke University Center for Human Genetics, Durham, NC.

Objective:To determine if family history of mood disorders and level of language ability impacts parent report of mood symptoms in children with Autism Spectrum Disorders(ASD). **Design/Methods**:Subjects were drawn from a genetic study of ASD,(n=187 Autistic Disorder(AD); n=46 Aspergers Disorder(AS)). Diagnoses were confirmed by medical records, clinical evaluation, the Aspergers Syndrome Diagnostic Scale and the ADI-R. ADI-R Question 19 was used to group by level of language. The variables were sleep problems and family history of mood disorder from the research chart, and Hyperactivity(ABC-H),Irritability(ABC-I), and Inappropriate Speech(ABC-IS)subscale scores from the parent completed Aberrant Behavior Checklist-Community(ABC-C). AD and AS group analyses were performed separately. **Results and Conclusions**: *AD Group*: Using Discriminant Function Analysis(DFA), sleep difficulties, ABC-H and ABC-I scores were combined to create a new factor of irritable mood symptoms(IMS). Factor loadings were: ABC-I=.588, ABC-H=-.157, and sleep difficulties=.156. There was no main effect for family history with 2x2 ANOVA. There was a significant main effect for speech ($F(1,186)=9.0869$, $p=.003$). These findings suggest parents rate verbal children with AD higher on the IMS factor than nonverbal children, and speech is important in parents ability to recognize mood symptoms. *ASD Group*: Using DFA, sleep difficulties, the ABC-H and ABC-I scores, and two questions from the ABC-IS scale on the ABC-C were combined to create a new factor of mood related symptoms (MRS). The factor loadings were: ABC-H=.915, ABC-I=.721, ABC-IS=.678 and sleep difficulties=.405. There was a significant main effect for family history by one-way ANOVA ($F(1,45)=4.487$, $p=.04$). Children with ASD and a family history of mood disorders may be at a higher risk of having mood related symptoms than those children without a family history of mood disorders. Further investigation of the potential role for mood symptoms in ASD is needed.

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Lissencephaly with marked pontocerebellar hypoplasia: clinical and molecular analysis of two affected sisters. *N. Ishihara[1,2], K. Yamada[2], Y. Yamada[2], Y. Okumura[3], M. Futamura[3], N. Wakamatsu[2].* 1) Deparment of Pediatrics, Kamo Hospital, Toyota, Aichi, Japan; 2) Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi, Japan; 3) Department of Neonatology, Central Hospital, Aichi Human Service Center, Kasugai, Aichi, Japan.

Lissencephaly is a group of clinically and genetically heterogeneous brain malformations characterized by a smooth cerebellar surface and agyria with or without pachygyria. Here, we present two affected sisters with a rare type of lissencephaly accompanied by marked pontocerebellar hypoplasia. One patient was a Japanese female born from healthy non-consanguineous parents. She was born at 39 weeks and 5 days gestation without any complications. At birth, her weight was 2936 grams and she had microcephaly (head circumference was 29.5 cm). Her APGAR score was 9 after 1 minute. When she was transferred to our hospital, myoclonus was observed in her face and body. Multifocal partial seizures occurred frequently, which were uncontrollable with anticonvulsants. She died 41 days after birth. The other patient was her sister born at 37 weeks and 6 days gestation. She also presented with a similar clinical course and died 36 days after birth. Brain CT and MRI of both patients showed lissencephaly without thickening of the cortical gray matter and severe pontocerebellar hypoplasia, but the cerebral atrophy was more severe in the younger patient. They also had ocular nerve atrophy. The high-resolution G-banded karyotype of both patients was normal. We performed a Southern blot analysis using cDNA of known causal genes of lissencephaly, such as *LIS1* and *RELN*, as probes but no deletion was found in either case. Sequence analysis of all exons and exon/intron boundaries of *RELN*, *DAB1* and *POMT1* revealed no gene mutations. These results indicate that the disease was likely caused by the mutation of a gene not yet identified as a cause of lissencephaly.

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Autosomal Recessive Lower Motor Neuron Disease with Childhood Onset: Clinical study and genetic mapping to chromosome 1p36.3 in an African family. *I. Maystadt[1], D. Leclair-Richard[2], B. Estournet[2], A. Barois[2], F. Renault[3], M.C. Routon[4], M.C. Durand[5], A. Munnich[6], C. Verellen-Dumoulin[2], L. Viollet[2,6].* 1) Centre de Génétique Humaine, Université Catholique Louvain, Bruxelles, Belgium; 2) Service de Neuropédiatrie, Hôpital Raymond Poincaré, Garches, France; 3) Service d'Explorations Fonctionnelles, Hôpital Trousseau, Paris, France; 4) Service de Neuropédiatrie, Hôpital Saint Vincent de Paul, Paris, France; 5) Service d'Explorations Fonctionnelles, Hôpital Raymond Poincaré, Garches, France; 6) INSERM U393. Génétique Médicale, Hôpital Necker Enfants Malades, Paris, France.

Lower Motor Neuron diseases (LMND) form a wide spectrum of rare sporadic and hereditary neuromuscular disorders, characterized by progressive degeneration of the motor neurons of the spinal cord. We reported here the clinical picture of a severe familial childhood onset LMND in a large inbred African pedigree, with autosomal recessive inheritance. First symptoms occurred at the age of 2 to 3,5 years. At the age of 5 years, most of the patients presented a real Gowers sign, a symmetrical atrophy of scapular muscles, bilateral equinus feet and retracted fingers Facial muscles were preserved. All tendon reflexes were abolished and diagnosis of motor neuron disease was suspected on neurological assays, showing muscle denervation with normal motor and sensory nerve conduction velocities. Conversely to classical childhood onset spinal muscular atrophies, no predominance of weakness between proximal and distal muscles was noted. The generalized muscle atrophy rapidly worsened and walking ability was lost at the age of 7,5 to 8,5 years. Severe scoliosis with hyperlordosis occurred during childhood and respiratory function progressively decreased, requiring assisted ventilation by tracheotomy at the age of 15 to 17 years. Genetic analysis in this pedigree allowed us to exclude the SMN gene (5q13.3) and to map the causing mutation in a 3,9 centimorgan interval between loci D1S508 and D1S2633 on chromosome 1p36.3 (Zmax=3,79 at locus D1S253). This study broadens the spectrum of hereditary childhood onset LMNDs and opens the way towards the identification of a novel gene involved in motor neuron degeneration.

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Meckel-Gruber syndrome in a fetal case with a 1p35-pter deletion and a 14q32-qter duplication inherited from a maternal balanced translocation. *C. Thauvin-Robinet[1], P. Callier[2], N. Laurent[3], N. Marle[2], T. Rousseau[4], P. Sagot[4], F. Huet[1], L. Faivre[1], F. Mugneret[2].* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Laboratoire de Cytogenetique, CHU Le Bocage, Dijon, France; 3) Laboratoire d'Anatomie-Pathologie, CHU Le Bocage, Dijon, France; 4) Maternite, CHU Dijon, France.

Meckel-Gruber syndrome is an autosomal recessive disorder characterized by a combination of renal cysts and variably associated features including developmental anomalies of the central nervous system (typically occipital meningoencephalocele), hepatic ductal dysplasia and cysts, and polydactyly (postaxial in most cases). Additional anomalies (microphtalmia, cleft palate, ear defects, cardiac, intestinal, and genital anomalies) are frequent. In few cases, there is a only a binary combination of either encephalocele or polydactyly with polycystic kidneys. Genetic heterogeneity has been demonstrated by the identification of three loci on chromosomes 8q24, 11q13 and 17q21-24. We report on a fetal case (15 WG) with cleft palate, encephalocele, bilateral club feet, enlarged bladder and multicystic kidneys, compatible with Meckel-Gruber syndrome. Liver histology could not be studied because of autolysis. Fetal chromosome analysis showed a maternally inherited unbalanced translocation between the short arm of chromosome 1 and the long arm of chromosome 14, resulting in 1p35-pter deletion and 14q32-qter duplication (46,XY,der(1),t(1;14)(p35,q32). Since chromosomal breakpoints have not been previously implicated in Meckel-Gruber syndrome, this observation would be interesting for the identification of other genes responsible for Mecker-Gruber syndrome or encephalocele.

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Psychiatric phenotype in Triple X Syndrome. *O. Caluseriu[1,2], E.W.C. Chow[2,3], A.S. Bassett[2,3].* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) University of Toronto, Toronto, ON, Canada.

Triple X Syndrome is a chromosomal abnormality with a variable pattern of CNS involvement including learning difficulties and psychiatric illness in adults (Harmon, 1998). Psychotic features are not considered part of the phenotype, although sex chromosome anomalies may have an increased prevalence in schizophrenia (SZ) (Bassett et al, 2000). We present two adult women with Triple X Syndrome and psychosis: one with full syndrome and one mosaic. Patient 1, was 31 y old, of Caucasian origin, ambidextrous, with minimal physical anomalies and history of ventricular septal defect, atrial septal defect, patent ductus arteriosus, delayed puberty, hypothyroidism, ovarian cysts, learning disabilities, alcohol and drug abuse. She had a history of psychotic symptoms (visual and auditory hallucinations) starting at age 10 y, and later onset of depression and self-injurious behavior. She was formally diagnosed with schizoaffective disorder and treated at age 28 y. The karyotype was 47, XXX and FISH for 22q11.2 deletion was negative. Patient 2, was 43 y old, of Chinese origin, with asymmetric face, borderline MR and dysarthria. She was diagnosed with SZ at age 25 y. She had no history of seizures although EEG suggested subcortical white and cortical grey matter dysfunction in both lateral fronto-temporal areas. The karyotype showed 47, XXX [33]/46, XX [23] in lymphocytes and a ratio 1:3 in favour of the triploid line from buccal swab. A possible association between schizophrenia and sex chromosome aneuploidies is difficult to interpret given the random pattern of X inactivation and mosaicism. Genetic variation (e.g., HOPA polymorphisms), imprinting disruption (Latham, 2005), gene dosage effects and molecular regulatory processes affecting gene expression (Birchler et al, 2005) are possible mechanisms for the implication of sex chromosomes aneuploidies in the susceptibility to SZ.

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Angelman syndrome from infancy to old age. *M. Philippart[1], B. Minassian[2].* 1) Semel Institute for Neuroscience and Human Behavior, Mental Retardation Research Center, UCLA, David Geffen School of Medicine, NPI C9-848,760 Testwood Plaza, Los Angeles, CA.90024-1759; 2) The Hospital for Sick Children, University of Toronto, 555 University Ave, Rm 6536B, Toronto ON M5G 1X8 Canada.

Twenty cases (14 females, 6 males, aged 2 to 74 years) of Angelman syndrome were followed for an average of 10 years (1-21). Thirteen had 15q11-13 deletions, 2 uniparental disomy, 2 UBE3A mutation, one inverted dup (15) marker, and two no anomalies. Psychomotor level was around 8-15 months, stable for up to 40 years. CT scans were normal in 8; 3 had mild ventriculomegaly and 3 cerebellar atrophy. The youngest were overweight. Behavior problems were uncommon. Seizures first occurred from 1 to 3 years of age. The oldest patient (15q11-13 deletion case) never had seizures. CCTV-EEG telemetry revealed epileptiform discharges in three who had normal EEGs, never had clinical seizures. Most had 1.5 to 2.5 Hz spike and waves. Most were tried on multiple AEDs. While Valproate was sometimes effective it caused problems in water balance, plasma albumin and osmolality, low blood glucose, without identified endocrine anomalies. Two developed heart failure with cardiomegaly. The younger children resembled Rett syndrome without failure to thrive and Prader-Willi with less hypotonia. Their exuberant personality was a main asset, leading to overestimation of social skills and tolerance of hyperactivity. Hand function was limited to simple manipulations; none could perform a 2-step command. Tremor was common. Ambulation remained precarious until age 61 in one and was lost in many older patients. Dystonia was minimal before middle age; 2 had Parkinsonism precipitated by Metoclopramide. Gastrointestinal problems were common. Happiness is the hallmark recognized by Angelman. Exuberance better describes their emotional state, giving them a drive to cope without the usual behavior difficulties commonly associated with mental retardation. The term puppet alluded to motor immaturity at the toddler level, the highest milestone attained in these patients. Clinical variability is wide within a narrow range of developmental skills throughout life.

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A Japanese case of novel immunodeficiency-malformation syndrome. *N. Norimatsu[1], T. Kondoh[1], Y. Uehara[2], N. Miyake[1], M. Moriuchi[1], N. Tominaga[1], K. Tsuruda[3], H. Kanegane[4], K. Tashiro[5], S. Kamihira[3], H. Moriuchi[1].* 1) Depts of Pediatr, Nagasaki Univ Hosp, Nagasaki, Japan; 2) Dept of Pediatr, Nagasaki Memorial Hosp, Nagasaki, Japan; 3) Dept of Lab Med, Nagasaki Univ Hosp, Nagasaki, Japan; 4) Dept of Pediatr, Toyama Med Pharm Univ, Toyama, Japan; 5) Ctr for Mol Biol Genet, Kyoto Univ, Kyoto, Japan.

Congenital immunodeficiencies are occasionally associated with abnormalities in other organ systems. We here report a novel immunodeficiency and multiple anomalies in a 15-year-old Japanese boy. At birth, his brain computed tomography did not show any abnormality, and height, weight and head circumference were normal, but he developed severe and progressive growth retardation and microcephaly thereafter. Since 18 months old, he has had recurrent respiratory and skin infections. He underwent left lung resection because of refractory infection at 10 years of age. The histological study of the hilar lymph nodes revealed the absence of B-lymphocytes and primary/secondary follicle formation. Flow cytometric analyses revealed markedly reduced CD19+ cells in the peripheral blood in contrast with substantial number of CD19+ cells in the bone marrow. In addition to severe short stature and microcephaly described above, he also had multiple anomalies such as characteristic facies, multiple arythrogryposis, severe mental retardation, epilepsy, progressive brain atrophy, congenital bilateral cataract, progressive optic nerve atrophy, retinitis pigmentosa, Fanconi syndrome, micropenis, severe osteoporosis, and prolonged reaction of primary teeth. His chromosomal tests revealed normal karyotype, 46, XY. FISH analysis in 22q11.2 and OCLS regions as well as sequencing of his BTK, Oct-2 and OBF-1 genes revealed no abnormality. Functional expression of CXCR4 and SDF-1 was normal. Our case is clearly distinct from immunodeficiency-malformation syndromes previously reported, particularly in B-cell defect characterized by a lack of recruitment of mature B cells from the BM to periphery, as well as a combination of systemic involvement. We are currently investigating molecular mechanisms of the pathogenesis of this patient.

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Correlation of phenotype and expression of four genes between breakpoints BP1 and BP2 in Prader-Willi syndrome. *M.G. Butler, N. Kibiryeva, D.C. Bittel.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is a genetic disorder that results from loss of paternally expressed genes from the 15q11-q13 region. The major features include hypotonia, mental deficiency, behavior problems, hypogonadism and hyperphagia leading to obesity. A paternal 15q11-q13 deletion is found in about 70% of PWS subjects, maternal disomy 15 (UPD) in 25% and an imprinting defect in 3%. The typical deletion is classified as Type I (TI) or Type II (TII) depending on involvement of two proximal breakpoints (BP1 or BP2). The TI deletion involving BP1 is 500 kb larger than the TII deletion involving BP2. We previously reported that TI individuals generally had a more severe phenotype compared with TII or UPD subjects, such as greater self-injury and obsessive-compulsive behavior, deficits in adaptive behavior, reduced reading and math skills and poorer visual integration. Four genes (*NIPA1, NIPA2, GCP5, CYFIP1*) located between BP1 and BP2 are deleted in TI individuals and implicated in poorer outcomes. We examined the expression of these genes by quantitative RT-PCR in eight TI PWS subjects (4 male, 4 female) and nine TII PWS subjects (3 male, 6 female) and found reduced but detectable expression in TI compared to TII subjects supporting biallelic expression. Correlations between gene expression C_T values and multiple behavioral, cognitive and visual integration measurements showed overlapping correlations of expression levels specifically with *NIPA2* [e.g., significant positive correlations between *NIPA2* expression and: adaptive behavior (SIB social interaction and communication index, $r = 0.83$, $p < 0.001$); cognitive ability (Woodcock-Johnson reading and math clusters: $r = 0.57$, $p = 0.02$ and $r = 0.58$, $p = 0.02$, respectively); visual-motor integration (VMI raw and standard scores, $r = 0.61$, $p = 0.01$ and $r = 0.65$, $p = 0.007$, respectively)]. *NIPA2* is conserved in vertebrates and widely expressed with transmembrane domains suggesting receptor or transporter function. Our data suggest that *NIPA2* may be important in influencing neurodevelopment and function deserving further study.

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A Controlled Family Study of Attention Deficit Hyperactivity Disorder and Gilles de la Tourettes Syndrome. *E. Stewart[1], C. Illmann[1], D. Geller[1], J. Leckman[2], R. King[2], D. Pauls[1].* 1) Psychiatry, Harvard Medical School, Charlestown, MA; 2) Psychiatry, Yale University, New Haven, CT.

Objective: Although ADHD is commonly comorbid with GTS, it is unclear whether these disorders have a common genetic etiology. This study examines familial relationships between DSM-IV GTS and ADHD in mutually-exclusive groups of GTS+ADHD, GTS-only, ADHD-only and controls. **Method:** This was a direct-interview, case-control family study of 692 first-degree biological relatives of 75 GTS+ADHD, 74 GTS-only, 41 ADHD-only and 49 control probands. Age-corrected prevalence rates, odds ratios and comorbidity patterns of GTS, ADHD and OCD among relatives were estimated from blinded best-estimate diagnoses. **Results:** GTS and ADHD were more prevalent among relatives of probands with those specific disorders versus control relatives (p0.001). In the GTS-only group, although ADHD occurred at higher than control rates (p=0.03), ADHD-GTS in relatives (p=0.82) was not increased. In the ADHD-only group, GTS-ADHD was not increased (p=0.18). ADHD+GTS rates in relatives were elevated in all case groups (p0.03). Comorbid GTS predicted ADHD in relatives (p<0.001), comorbid ADHD predicted GTS in relatives (p<0.001) and comorbid OCD predicted both ADHD (p=0.002) and GTS (p<0.001) in relatives. **Conclusions:** Relatives of individuals with either ADHD or GTS are at increased risk for those same disorders in addition to comorbid ADHD+GTS. In familial forms of ADHD and GTS, their comorbid presence among relatives appears to be etiologically related rather than coincidental. However, the rates of GTS and ADHD alone are not consistent with the hypothesis that the same etiologic factors are acting in both disorders. Comorbid OCD may be a useful marker of ADHD cases related to GTS.

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Microarray analysis in a second reported case of 18p11.2 monosomy in a psychotic patient. *C.M. Drazinic[1], B.A. Pletcher[3], H. Zheng[1], M.W. State[1,2].* 1) Child Study Center, Yale University, New Haven, CT; 2) Department of Genetics, Yale University, New Haven, CT; 3) Center for Human and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ.

Microarrays provide an efficient way to map breakpoints in patients with chromosomal copy number abnormalities. In a recent report, microarrays were used to map 18p11.23 monosomy and 5p14.1 trisomy breakpoints in a female with dysmorphic features, short stature, mental retardation, and psychosis (Drazinic et al., 2005). Here we describe a second female with isolated 18p monosomy, who also presents with dysmorphic features, short stature, mental retardation, and psychosis. The patient's genomic DNA was analyzed using a GeneChip Human 50K XbaI 240 Array (Affymetrix), consisting of 58,960 single nucleotide polymorphism (SNP) probes with an average spacing of 46.5 kilobases. Fluorescence in situ hybridization (FISH) confirmed the deletion, using probes from bacterial artificial chromosomes (BAC's) of an RP-11 library. The patient's G-banded karyotype was read as 46, XX, del(18)(p11.2), with a deletion similar to the 18p monosomy derivative chromosome described in the previous report. Using the 50K SNP array, the 18p deletion breakpoint in this case mapped telomeric to the 14.9 megabase position, in band 18p11.21 (May 2004 freeze; http:genome.ucsc.edu). This breakpoint maps approximately 0.5 Mb away from the centromeric band 18p11.1, which begins at 15.4 Mb (May 2004 freeze; http:genome.ucsc.edu). While microarrays can be used to rapidly approximate the breakpoints in patients with unbalanced karyotypic abnormalities, these cytogenetic variations need to be confirmed with alternative methods such as FISH. Case reports and linkage analyses have implicated the short arm of chromosome 18 in patients with psychosis. The presence of two independent cases with both 18p11.2 monosomy and psychosis suggests that further investigations need to be conducted to determine the relative contributions of candidate genes on 18p to the psychosis phenotype.

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Natural History of Aicardi Syndrome. *V.R. Sutton[1], M.A.K Glasmacher[2], T.N. Eble[1], B.J. Hopkins[1], R.A. Lewis[1], D. Park Parsons[2], I.B. Van den Veyver[1].* 1) Baylor College of Medicine, Houston, TX; 2) Aicardi Syndrome Newsletter, Louisville, KY.

Aicardi syndrome is a rare neurodevelopmental disorder characterized by agenesis of the corpus callosum, other developmental brain abnormalities, chorioretinal lacunae and severe seizures. Current clinical knowledge is derived from small case series that focus on these major defects. A detailed questionnaire covering information on general health, organ systems and family history was sent to 216 families of children with Aicardi syndrome to expand our knowledge of this disorder and to uncover previously unrecognized features of Aicardi syndrome. Of the 80 completed returned surveys, 75 met inclusion criteria and were further analyzed. The ages of children with Aicardi syndrome ranged from 5 months to 32 years (mean: 86 months). There was one boy with a 47,XXY karyotype, all others were girls. The growth in Aicardi syndrome is normal during early childhood and slows between ages 7 (weight) and 9 (height) with final weight and height reaching a mean at the 5th centile with a normal body mass index (BMI). Growth curves superimposed on standard CDC growth curves were constructed and will be presented. A Kaplan-Meier survival curve is presented and reveals the median age of survival was 18.5 years, which is more favorable than previously reported. We confirmed the high prevalence of chorioretinal lacunae (96%), agenesis of the corpus callosum (95%) and severe complex seizures (99%). There was a wide variety of seizure types and seizures were generally very difficult to control with a large range of medications and therapies having been tried. Vigabatrin or a vagus nerve stimulator provided the most satisfactory seizure control although most individuals were on multiple medications and no antiepileptic monotherapy was clearly the best treatment. The most common complication after seizures was gastrointestinal dysfunction, present in >90%. The results from this survey contribute new information on Aicardi syndrome that will benefit clinical management and patient counseling. The collected data are also of value for phenotype-driven research towards its underlying cause.

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Candidate gene analysis in 4 families with CDAGS syndrome (craniosynostosis, delayed fontanel closure, cranial defects, anal and genital anomalies and skin eruption) mapping to chromosome 22q. *R. Mendoza-Londono[1, 2], D.A. Scott[1], E. Lammer[3], R. Watson[4], A. Hatamochi[5], D. Napierala[6], P. Hermanns[1], B.B. Roa[1], M.R. Hegde[1], D.W. Stockton[1], B. Lee[1, 6].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 3) Childrens Hospital Research Institute, Oakland, CA; 4) Our Ladys Hospital for Sick Children Crumlin, Dublin, Ireland; 5) Dept. of Dermatology, Dokkyo University School of Medicine, Japan; 6) Howard Hughes Medical Institute, BCM, TX.

CDAGS syndrome is a rare genetic condition characterized by craniosynostosis, delayed closure of the fontanel, cranial defects, clavicular hypoplasia, anal and genitourinary malformations and skin eruption. We have identified 7 patients in 4 families from different geographic regions and ethnic backgrounds with this phenotype. This is an autosomal recessive condition that brings together apparently opposing pathophysiologic and developmental processes, including accelerated suture closure and delayed ossification. Selected candidate genes including RUNX2, CBFB, MSX2, ALX4, TWIST1, and RECQL4 were screened for mutations by direct sequencing of their coding regions, and for microdeletions by FISH. No mutations or microdeletions were detected in any of the genes analyzed. A genome wide screen yielded the maximum estimated LOD score of +2.38 for markers D22S283 and D22S274 on chromosome 22q12-q13. Expression microarray analysis was used to identify candidates for differentially expressed genes between fibroblast from affected individuals compared to normal controls. Genes located in the area of linkage that were significantly down regulated were sequenced. To date we have excluded mutations in the coding regions of RBM9, DDX17 and ATF4. We hypothesize that the gene defect in CDAGS exerts novel context-dependent regulation of multiple signaling pathways, including RUNX2 during osteoblast differentiation and craniofacial morphogenesis.

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Hereditary neuropathy with liability to pressure palsies: clinical manifestations do not fit the name. *M. Sabatelli[1], M. Zollino[2], M.L. Mereu[1], F. Madia[1], A. Conte[1], G. Loria[1], R. Lecce[2], G. Neri[2], P. Tonali[1].* 1) Neurologic Institute, Catholic University, Rome, Italy; 2) Medical Genetics Institute, Catholic University, Rome, Italy.

Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominantly inherited disease of peripheral nerves associated with a 1.5-megabase deletion on chromosome 17p11.2-12 containing the PMP22 gene. Recurrent mononeuropathies manifesting as transient episodes of weakness or sensory loss, often preceded by minor trauma, are considered the clinical hallmark of this condition. However a minority of patients have been described disclosing atypical phenotypical pictures, including symmetric chronic polyneuropathy, brachial plexopathies, Guillain-Barrè syndrome, chronic mononeuropathies. With the purpose of characterizing the phenotype of HNPP we evaluated both the clinical picture and neurophysiological findings in 34 patients from 24 unrelated families, disclosing chromosome 17p11.2-12 deletion. A total of 11 patients (32%) presented with classic episodes of transient mononeuropathy related to trivial nerve trauma. In the remaining 23 patients (68%) the clinical picture was different, including chronic mononeuropathy (4 carpal tunnel syndrome and 5 ulnar compression at the elbow) in 9 patients, a Charcot-Marie-Tooth phenotype in 7, a Guillain-Barrè-like pattern in one patient, chronic sensory polyneuropathy in 2. One patient presented with generalized weakness, another one with acute recurrent brachial plexopathy. The last 2 patients were asymptomatic. In all patients electrophysiological studies showed generalized neuropathy with moderately slowed conduction velocities, more obvious over entrapment sites. Our data show that the neurological phenotype associated with 17p11.2 deletion is very heterogeneous and that classic recurrent focal neuropathy, largely accepted as distinctive of this condition, is not the most frequent clinical presentation. Peculiar electrophysiological findings appear to be more reliable than clinical examination for selection of patients to be tested genetically.

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Neurofibromatosis -1 is associated with a high incidence of migraine in both sexes. *K. Gardner[1,2], C. Roche[2], P. Duzenli[2], M. Amjhad[2], A. Goldstein[2], G. Alper[2], C. Kammerer[3].* 1) Dept Neurology, Veteran's Administration Pittsburgh Healthcare System; 2) Dept Neurology, Children's Hospital of Pittsburgh; 3) Dept Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Background: Headache (HA) is a well-recognized complaint in NF1 patients. Prior studies conflict regarding frequency of HA's in Neurofibromatosis 1 (NF1). One study suggested a reverse incidence of migraine with aura (MA) and migraine without aura (MOA) and more tension than migraine. Another study suggested a lower than expected incidence of all headache types; both studies used modified 1988 International Headache Society (ICHD-I) diagnostic criteria for HA and/or relied partially on questionnaires. Methods: We evaluated 70 probands and 88 total subjects from an NF clinic and confirmed NF1 diagnosis using the 1997 NIH consensus criteria. We used a structured, validated interview to note all headache features and types according to ICHD-I criteria. General and neurological exams, scans, and medical records were reviewed to identify nonprimary headache causes with questionnaire for headache triggers, etc. Results: Fifty-six percent (0.560.05) of probands (24/43 females and 15/27 males, p>0.95 sex difference) had migraine compared to a general population prevalence 16.5% while 19 percent (8/43 females and 5/27 males) had migrainous headache not meeting criteria, I.H.S. 1.7. Eighteen females and 8 males had episodic tension (TN) with one or more migrainous features and only 5 females and 1 male had typical episodic TN. There were 20 females and 9 males with 1 HA type, 14 females and 10 males with 2 HA types, 5 females and 3 males with 3 HA types, 2 females and 1 male with 4 HA types, 2 females with 5 HA types. Overall 34 percent had MOA (10 males, 14 females) and 20 percent had MA (4 males, 10 females) as their most severe HA. Females had 3 and males 1 secondary headache, not all tumor related. Conclusion: Migraine prevalence is higher than expected and equal in both sexes among NF1 clinic patients compared to general population prevalence studies. Multiple headache types are common and most have one or more migrainous features, including those meeting criteria for episodic tension.

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Macrocephaly-cutis marmorata telangiectatica congenita: Further delineation of a clinically distinctive overgrowth syndrome. *J.T.C. Shieh, H.E. Hoyme.* Division of Medical Genetics, Department of Pediatrics, Stanford University, Stanford, CA.

Macrocephaly-cutis marmorata telangiectatica congenita (M-CMTC, OMIM 602501) is a syndrome characterized by macrocephaly, overgrowth, cutis marmorata, facial nevus flammeus, syndactyly or polydactyly, and hydrocephalus. We describe a patient with the key features of M-CMTC who has linear ear creases and an adrenal mass. The patient was born at 39 weeks gestation with a head circumference of 37cm and weight of 3685g. In the newborn period, mottling of the extremities prompted an echocardiogram and a head CT scan, both of which were unrevealing. At 2 months of age, the patient was referred for genetics evaluation for facial angiomata. She had macrocephaly, cutis marmorata, bilateral linear ear creases, facial dysmorphism, and hypotonia. A faint reticular purplish patch was present on the right half of the face, and hemangiomas were present on the back. At 5 months of age, an MRI revealed hydrocephalus and CSF shunting was performed. Additional findings included small hands, loose skin and lower extremity asymmetry. A diagnosis of M-CMTC was made. Because of a potential risk of malignancy in this overgrowth syndrome, the patient underwent screening abdominal ultrasounds, and a soft tissue mass was detected. Subsequent MRI demonstrated an adrenal mass that is currently being investigated. M-CMTC is of unclear etiology and pathogenesis. In the index patient, high resolution chromosome analysis and FISH for subtelomeric rearrangements were normal. Methylation analysis of H19 and LIT1 were unremarkable. FISH for NSD1 deletion was normal. M-CMTC is a clinically distinctive disorder, and the presence of linear ear creases contributes a new finding to the M-CMTC phenotype. Our case supports the need for patients with M-CMTC to be screened for potential malignancies. Further studies, however, are needed to elucidate the genetic etiology underlying this disorder.

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Cognitive and language deficits among children with chronic neuronopathic Gaucher disease. *R. Schiffmann[1], O. Goker-Alpan[2,3], E. Wiggs[1], M. Eblan[2,3], E. Sidransky[2,3].* 1) DMNB/NINDS, NIH, Bethesda, MD; 2) NSB/NIMH, NIH, Bethesda, MD; 3) MGB/NHGRI, Bethesda, MD.

The advent of enzyme replacement therapy (ERT) has decreased mortality and morbidity in Gaucher disease(GD). Although ERT has resulted in fewer systemic complications, neurologic involvement is not impacted. Chronic neuronopathic (type 3) GD presents with diverse neurologic features between infancy and early childhood. We reviewed cognitive and speech testing in 32 children with type 3 GD on ERT. Diagnostic screening for mutations identified L444P/L444P as the most common genotype, and other mutations included G377S, N188S, and recombinant alleles. Wechsler Intelligence Scales demonstrated discrepancies between verbal and full scale IQ/DQ, with higher verbal IQ scores and weaknesses in processing speed, visual-spatial and perceptual organization skills. This gap increased upon sequential testing, with more gains in verbal areas. Despite relative verbal strengths, speech delays, especially expressive, frequently occurred. More than 60% of subjects had below average intellectual skills, and 13% had severe impairment. The degree of intellectual deficits concurred with neurologic involvement, and patients with recurrent seizures and ataxia received the lowest scores. Cognitive decline occurred in four with myoclonic epilepsy, and two were diagnosed with autism. On ERT, the systemic manifestations were usually mild, and no correlation was observed between the IQ scores and residual enzyme activity or genotype. While cognitive and speech problems are common in children with type 3 GD, the degree of impairment may be under-recognized, as the deficits are often compensated by verbal strengths. The visual-spatial weaknesses and cases of autism may correlate with the known hippocampal and parietal cortex involvement in GD. Neurodevelopmental screening should be part of routine medical care in GD, and remediation strategies for these patients might focus on their verbal strengths.

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SCA17 in Italy: A molecular study in a large cohort of familial and sporadic ataxic patients. *C. Gellera[1], R. Fancellu[1], A. Castucci[1], G. Giaccone[2], P. Soliveri[3], F. Taroni[1], S. Di Donato[1], C. Mariotti[1].* 1) Dept. of Biochemistry & Genetics, Istituto Neurologico Besta, Milano, Italy; 2) Dept. of Neuropathology, Istiuto Neurologico Besta, Milano, Italy; 3) Dept. of Neurology, Istituto neurologico Besta, Milano, Italy.

Autosomal dominant spinocerebellar ataxias (ADCAs) are a heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar ataxia associated with other neurological features. At present, more than twenty SCA (spinocerebellar ataxia) loci have been identified and twelve disease-genes. In 7 disease-genes the molecular lesion is an expanded CAG repeat sequence within the coding region. SCA17 is caused by a CAG/CAA expansion in the gene encoding the TATA-binding protein (TBP). We screened for SCA17 expansion 174 subjects with cerebellar ataxia, including 34 sporadic and 140 familial cases. The cases presenting an autosomal dominant trait (n=96), repeat expansions in the SCA1, 2, 3, 6, 7 have been excluded. An additional group of 79 subjects with suspected Huntington disease, who were negative at the molecular test has also been screened. Expanded allels with >= 45 CAG repeats were found in 11 affected individuals and in three presymptomatic subjects from 7 unrelated Italian families. Two patients were sporadic cases, four patients (2 families) had familial spinocerebellar ataxia, while 5 patients (3 families) had positive family history for movement disorder. Eight out of 14 subjects carried 45 CAG repeats, one patient carried 46 repeats, while 5/11 individuals had expansions larger than 50 repeats. All mutated subjects in each family carried the same size of CAG expansion, that resulted stable during parent to offspring transmissions. In our patients the age at onset ranged from 19 to 55 years. In 4/11 patients, the onset was characterized by cerebellar gait ataxia and limb incoordination, while in the remaining cases, the onset was characterized by behavioral disturbances, psychosis or mild choreic movements. Our data confirm that CAG expansion in the TBP gene can be associated with variable clinical features ranging from Huntington disease-like phenotype to familial spinocerebellar ataxia.

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Clinical and molecular correlations in FXTAS. *F. Tassone[1], C. Greco[2], R. Berman[3], R.M. Martin[3], P.H. Schwartz[4], E.J. Becker[1], J. Papazian[5], S. Bacalman[5], L. Li[1], R.J. Hagerman[5], P.J. Hagerman[1].* 1) Department of Biochemistry and Molecular Medicine, University of California, Davis, School of Medicine, Davis, CA; 2) Department of Pathology, University of California, Davis, Medical Center, Sacramento, CA; 3))Department of Neurosurgery, University of California, Davis, School of Medicine, Davis, CA; 4) Childrens Hospital of Orange County Research Institute, Orange, CA; 5) M.I.N.D. Institute, University of California, Davis, School of Medicine, Davis, CA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurological condition, characterized by intention tremor, gait ataxia, parkinsonism, and cognitive decline. Brain imaging reveals generalized brain atrophy and white matter disease, particularly involving the middle cerebellar peduncles (MCP). Global cerebral atrophy, marked Purkinje cell dropout, and the presence of ubiquitin-positive neural intranuclear inclusions represent the main neuropathological features of FXTAS. Although it is clear that FXTAS is associated with premutation alleles, the precise relationship between the disease manifestations and the size of the repeat is not known. To address this issue, we have studied the association between CGG repeat number, FMR1 mRNA levels, and several clinical measures, including age of onset of symptoms, of ataxia, and of tremor, in a group of 38 male premutation carriers (mean age 59.4 6.6 year) affected by FXTAS. Statistical analysis showed a significant association between the number of CGG repeats and age of onset of symptoms (p=0.02), although no association was observed between age of onset of symptoms and FMR1 mRNA levels. Neuropathological studies, based on 11 males who died with FXTAS, showed a strikingly significant correlation (p0.01) between the number of intranuclear inclusions and the number of CGG repeats in several brain regions, including cortical gray matter and hippocampus. A significant correlation was also found between increasing number of CGG repeats and decrease in age of death (p0.01). Our findings suggest a role of the CGG repeat expansion as a prognostic indicator of both clinical and neuropathological involvement in FXTAS.

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Pre- and post-natal imaging of familial primary microcephaly with simplified gyral pattern and tentative linkage to *ASPM* (MCPH5). *M. Abramowicz[1], M. Cassart[2], C. Donner[3], P. Van Bogaert[4], J. Desir[1].* 1) Dept Genetics, Hosp Erasme - ULB; 2) Dept Radiology, Hosp Erasme - ULB; 3) Dept Obstetrics & Gynecology, Hosp Erasme - ULB; 4) Dept Pediatric Neurology, Hosp Erasme - ULB, Free University of Brussels, Belgium.

Autosomal recessive primary microcephaly (MCPH) is characterized by a congenitally small but normally formed brain, and nonprogressive mild-to-moderate mental retardation. It is genetically heterogeneous with at least seven loci, and four genes identified so far. It is unclear whether the phenotype described as primary microcephaly with simplified gyral pattern relates to mutations of the same genes as MCPH. We report on a consanguineous family of Morroccan origin who consulted during a second pregnancy for microcephaly detected at 26 weeks gestation. The first child, a girl aged 5 years, presented MCPH with a head circumference at -7SD, a normal physical examination, a normal psychomotor development, and a hyperactive behavior with moderate mental retardation. She had had two episodes of seizures in the past which were not treated. MRI of the brain showed a simplified gyral pattern with a clear anterior > posterior gradient, where the frontal lobes were small and squared-off. The fetus, a male, had two serial brain MRIs in utero which showed short and hypoplastic frontal lobes as in the proband sister, with diffuse albeit anterior > posterior simplification of the gyral pattern. At birth his HC was 31 cm, with an otherwise normal physical and neurological examination. A third, postnatal MRI showed persistance of the same brain anomaly. Microsatellite markers of the *ASPM* locus were found homozygous and concordant in both sibs, with no evidence of linkage to the reelin gene nor to other MCPH loci. To our knowledge this is the first pre- and postnatal imaging study of autosomal recessive microcephaly with simplified gyri, the first description of an anterior > posterior gradient in this condition, and its first tentative association with *ASPM*.

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The cholesterol transporter ABCG1 may contribute to the accelerated onset of Alzheimers Disease in Down Syndrome. *G.H. Tansley, B.L. Burgess, J. Chan, C.L. Wellington.* Pathology and Lab Medicine, UBC, Vancouver, BC, Canada.

Alzheimers disease (AD) is a neurodegenerative disorder affecting 80% of the population over 85 years of age. Extracellular deposits of aggregated amyloid beta peptides (A) are a neuropathological hallmark of AD, and factors that accelerate A production or impede its clearance may affect the onset or progression of AD. Intracellular cholesterol levels are known to regulate the production of A peptides. The cholesterol transporter ABCG1 is highly expressed in brain and maps to chromosome 21q.22.3 in the critical region for Down Syndrome (DS). Notably, individuals with DS typically manifest with AD in their 30s, suggesting that overexpression of genes such as ABCG1on chromosome 21 may accelerate AD pathogenesis. HEK cells stably expressing amyloid precursor protein (APP) containing the Swedish mutation (APPswe) were transiently transfected with an ABCG1 cDNA or empty vector. Cholesterol efflux assays were used to confirm ABCG1 activity, A peptides were measured by ELISA, and intracellular APP levels were determined by Western blot. Secretase activity was quantified by a fluorogenic assay. ABCG1 mRNA levels were determined in DS, AD, and control brain by quantitative PCR. We show that ABCG1 is overexpressed by 2-fold in DS frontal cortex compared to control and AD brain ($p<0.001$). HEK-APPswe expressing ABCG1 display a 1.2 fold increase in cholesterol efflux ($p<0.001$), confirming ABCG1 function. Moroever, cells expressing ABCG1 secrete 70% more A peptides within a 6h period compared to control cells ($p<0.001$). By 32h, approximately 3-fold more A peptides are secreted from ABCG1-expressing compared to control cells ($p<0.001$). Western blots show no difference in APP levels, indicating that the increase in A secretion cannot be accounted for by an increase in APP expression. Finally, both ABCG1-expressing cells and DS brain show a selective decrease in -secretase activity ($p<0.01$). Our results demonstrate that excess ABCG1 is present in DS brain and enhances A production. These observations suggest that ABCG1 may alter cholesterol metabolism in DS brain and contribute to the accelerated onset of AD neuropathology in DS individuals.

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A new case of Megalencephaly and Perisylvian Polymicrogyria with Postaxial Polydactyly and Hydrocephalus: MPPH syndrome. *M. Colombani[1], M. Chouchane[2], G. Pitelet[4], L. Morales[5], JP. Pinard[5], L. Lion-François[4], C. Thauvin[1], F. Huet[1], L. Guibaud[3], L. Faivre[1].* 1) Departement of genetics, children hospital, Dijon, France; 2) Departement of pediatrics, Dijon, France; 3) Departement of radiology, Debrousse hospital, France; 4) Departement of pediatrics, Debrousse hospital, France; 5) Departement of pediatrics, Beaune hospital, France.

We report a new case of polymicrogyria, polydactyly, hydrocephalus with mental retardation. This 1 year-old girl was first child of healthy non-consanguineous parents. When she was born at 39 WG, weight was 3670g (+3 DS), length 53cm (+2 DS) and OFC 37cm (+4 DS). Family history was unremarkable. Ultrasound scan at 24 WG showed mild ventricular dilatation with unique ombilical artery and dacryocystocele. Clinical examination showed post axial polydactyly at the right hand and both feet, and axial hypotonia with hypertonic arms and legs. Facial dysmorphism inclued macrocephaly with proeminent forehead, dacryocystocele, hypertelorism, telecanthus, depressed nasal bridge, bulbous nose, large jaws with a small mouth. Standart cytogenetic examination was normal. At 8 months of age, weight was +3 DS, length + 1.5DS and the OFC +4 DS. She could not sit because of an opisthotonos attitude, but had no major feeding difficulties. Contact was satisfying. Echocardiography and renal ultrasond were normal, but brain magnetic resonance imaging showed the persistance of ventricular dilatation and polymicrogyria predominantly on frontal region. Electroencephalogram did not show any seizures. Occular evoked potentials were normal whereas abnormal ear conduction was noted. Advanced bone age was significant (2 ½ years at 8 months of age). In conclusion, we report another observation with MPPH similar to the five patients described by Mirzaa and al. (Neuropediatrics 2004;35:353-359), although a predominantely anterior distribution of the polymicrogyria. From this observation, we can postulate that macrosomia at birth was found in 5/6 cases and might be a major feature of this syndrome. Also, dacryocystocele might be a rare associated feature. The mode of inheritance of this syndrome remains unknown since there was no significant family history.

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CDKL5/STK9 mutations in two unrelated females with early-onset infantile spasms. *M. Rio[1], D. Louesdon[1], N. Bahi-Buisson[2], P. Plouin[2], A. Munnich[1], J.P. Bonnefont[1].* 1) Department of Medical Genetics and INSERM U393, Hosp Necker-Enfants Malades, Paris, France; 2) Department of Neuropediatrics, Hosp Necker-Enfants, Paris, France.

Truncating and missense mutations of the X-linked cyclin-dependent kinase-like 5 gene (CDKL5/STK9) have been recently shown to cause a severe phenotype of early-onset infantile spasms, global developmental arrest, and severe mental retardation or a Rett syndrome-like phenotype (Kalscheuer et al. 2003, Weaving et al. 2004 and Tao et al. 2004). Here, we report two unrelated females with severe early-onset infantile spasms ascribed to CDKL5 mutations. The two girls developed infantile spasms in the first month of life after a normal pregnancy and delivery. Both patients had normal growth parameters for height, weight and head circumference. They did not show stereotyped hand movements or hyperventilation episodes. Severe psychomotor retardation was noted during infancy but their clinical course was different thereafter. Indeed, patient 1, now aged 3.5 years, showed generalized hypotonia and motor retardation in the first months of life. Seizures discontinued at 20 months but her development was very slow, and she was unable to sit unaided at 3.5 years. Sequencing analysis of CDKL5 coding regions showed a two bp deletion in exon 16 (c.2323-2324 del GA) leading to a stop codon at position 799 of the cDNA (p.E775fsX799). Patient 2, now aged 7 years , could sit unaided at the age of 16 months, started to walk at 3.5 years and say her first words at 2 years. Seizures remained very difficult to control and she developed intractable seizures during childhood. At 5 years of age, she showed developmental regression and lost her verbal and motor abilities. Sequencing analysis of CDKL5 coding regions showed a two bp deletion in exon 18 (c.2635-2636 del CT) leading to a stop codon at position 908 (pL879fsX908). These findings emphasize the extreme severity and precocity of infantile spasms, the absence of acquired microcephaly, and the broad variability of the clinical outcome associated with CDKL5 mutations.

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Brain MRI findings in a fœtus carrying an oligophrenin-1 (OPHN1) mutation and in his carrier mother. *F. Mochel[1], N. Boddaert[2], P. Sonigo[2], M. Rio[1], N. Philip[3], F. Brunelle[2], C. Beldjord[4], P. de Lonlay[5], A. Munnich[1], S. Lyonnet[1].* 1) Service de Génétique and INSERM U393, Hopital Necker-Enfants Malades, Paris, France; 2) Service de Radiologie Pédiatrique, Hopital Necker-Enfants Malades, Paris, France; 3) Département de Génétique, Hopital La Timone, Marseille, France; 4) Laboratoire de Génétique Moléculaire, Hopital Cochin, Paris, France; 5) Service des Maladies Métaboliques, Hopital Necker-Enfants malades, Paris, France.

The oligophrenin-1 gene (OPHN1) maps to Xq12 and encodes a Rho-GTPase-activating protein highly expressed in fœtal brain. OPHN1 mutations have been recently involved in X-linked mental retardation with cerebellar hypoplasia (Billuart et al 1998, Philip et al. 2003). Male patients usually present with psychomotor retardation of variable severity, hypotonia, epilepsy, awkward gait and strabismus in infancy. Minor facial dysmorphism has been also noted, including deep set eyes and a prominent chin (Philip et al. 2003). In all reported patients, brain MRI showed evidence of cerebellar abnormalities, namely large cisterna magna, cerebellar hypoplasia and/or cerebelloschisis (additional cerebellar fissure). Here, we report two affected brothers presenting with moderate psychomotor retardation (IQ=78), with infantile hypotonia and slight facial dysmorphism. One patient also had strabismus and EEG anomalies. Brain MRI of the affected sibs showed similar cerebellar abnormalities with medial vermian hypoplasia and cerebelloschisis. Brain MRI of their mother also evidenced diffuse vermian atrophy and cerebelloschisis. Interestingly, a foetal MRI performed at 30 weeks of pregnancy for the second sib retrospectively showed a large cisterna magna with partial vermian hypoplasia. Based on the association of mental retardation with vermian abnormalities in two male patients, we screened for mutations in OPHN1. Deletions of exons 16 to 19 were found in both patients, and in their phenotypically normal carrier mother. Therefore, brain MRI findings may help diagnosing OPHN1 mutations in male patients with mental retardation. The visualisation of such a MRI pattern in an affected foetus, as well as in an at risk mother, may also contribute to genetic counselling.

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Paraoxonase-1 Gly192Arg and Leu55 Met, and Paraoxonase-2 Ser311Cys polymorphisms and risk for sporadic Amyotrophic Lateral Sclerosis. *A. Slowik[1], B. Tomik[1], D. Partyka[1], W. Turaj[1], J. Pera[1], T. Dziedzic[1], D.A. Figlewicz[2], A. Szczudlik[1].* 1) Dept Neurology, Jagiellonian Univ, Krakow, Poland; 2) Dept Neurology, University of Michigan, Ann Arbor, MI.

INTRODUCTION: The human paraoxonase (PON) gene family consists of three members, PON1, PON2, and PON3, which are located adjacent to each other on chromosome 7. These enzymes protect LDL from oxidation and play a role in detoxification of organophosphate insecticides, pesticides, neurotoxins and arylesters. The activity of PON1 is modulated by Gln192Arg and Leu55Met polymorphisms, and PON2 by Cys311Ser polymorphism. Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disorder involving motor neurons in the brain and spinal cord. Epidemiological studies suggest that exposure to environmental toxins may be a risk factor for the development of sporadic ALS. AIM: We have investigated a possible association between PON1 and PON2 polymorphisms and the risk of sporadic ALS. METHODS: 168 patients with sporadic ALS and 437 healthy controls were studied. The definite or probable diagnosis of ALS was established according to El Escorial Criteria (1998). Paraoxonase polymorphisms were studied by PCR and restriction enzyme digestion. RESULTS: The Arg/Arg genotype of PON1 gene is overrepresented in ALS cases as compared to their controls [cases: Gln/Gln 47.6%; Gln/Arg 40.4%; Arg/Arg 12.0% vs. controls: Gln/Gln 54.9%; Gln/Arg 38.2%; Arg/Arg 6.9%, $p<0.049$]. Genotypes with Cys allele of PON2 gene are overrepresented in cases as compared to their controls (cases: Cys/Cys 10.2%; Cys/Ser 42.8%; Ser/Ser 47% vs. controls: Cys/Cys 6.9%; Cys/Ser 35.5%; Ser/Ser 59.6%; $p=0.005$). The significant differences between ALS cases and controls are also found in the PON1 Gln192Arg ($p=0.03$) and PON2 Cys311Ser ($p=0.004$) alleles distributions. The PON1 Leu55Met genotype and allele distributions do not differ between ALS cases and controls. CONCLUSIONS: PON1 Gln192Arg and PON2 Cys311Ser polymorphisms influence the risk for sporadic ALS. These results support the hypothesis that genotype-dependent differences in sensitivity to environmental chemicals may play a role in the development of ALS.

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A Novel PTPN11 mutation in an infant with Noonan syndrome (NS) and juvenile myelomonocytic leukemia (JMML). *G.C. Gowans[1], A. Asamoah[1], S.P. Ahuja[2], J.H. Hersh[1].* 1) Genetics Unit, Ped, WCEC, Univ Louisville, Louisville, KY; 2) Pediatric Heme-Oncology Unit, Ped, Univ Louisville, Louisville, KY.

Noonan syndrome (NS) is an autosomal dominant condition characterized by short stature, facial dysmorphism, a short and/or webbed neck, thorax deformations, heart defects and cryptorchidism in males. In 2001, missense mutations of the PTPN11 (protein tyrosine phasphatase, nonreceptor type 11) gene on chromosome 12 encoding protein tyrosine phosphatase (PTP) SHP-2 explained 30 - 50% of cases clinically diagnosed as NS. Mutations in PTPN11 have also been found to be present in LEOPARD/multiple lentigines syndrome, Noonan-like multiple giant cell lesion syndrome and cardiofacialcutanous syndrome. It has been previously reported that somatic mutations in PTPN11 account for 34% of nonsyndromic juvenile myelomonocytic leukemia (JMML). JMML has been rarely reported in NS. Germline mutations in PTPN11 have been found in most cases of NS with JMML. We describe an eleven month old Caucasian male infant with clinical features of NS and a mild case of JMML. Sequence analysis revealed the presence of a heterozygous single base change of C T in exon 13 of the PTPN11 gene (CCC CTC). This mutation is predicted to change the normal proline amino acid to a leucine at amino acid 491 of the protein. Mutations affecting this codon (P491S) have been reported previously. The P491L missense mutation is a novel mutation not previously reported for NS or JMML. We review the myeloproliferative and myelodysplastic conditions reported in NS.

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Prolonged survival in generalized arterial calcification of infancy is associated with hypophosphatemia and renal phosphate wasting. *F. Rutsch[1], B. Lorenz-Depiereux[2], N. Ruf[3], G. Ciana[4], Z. Mughal[5], C. Loirat[6], J. Davies[7], P. Nürnberg[8], T. Strom[2], D. Schnabel[9].* 1) University Children's Hospital, Muenster, Germany; 2) National Research Center for Environment and Health, Neuherberg, Germany; 3) Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; 4) Childrens Hospital, Trieste, Italy; 5) St. Marys Hospital Manchester, UK; 6) Hôpital Robert Debré, Paris, France; 7) University Hospital of Wales, Cardiff, UK; 8) Cologne Center for Genomics, University of Cologne, Germany; 9) Children's Hospital, Charité University Hospital, Berlin, Germany.

Generalized infantile arterial calcification (GACI, OMIM 208000) is caused by mutations of *ENPP1* encoding for E-NPP1 (EC 3.1.4.1). This cell surface enzyme regulates soft tissue calcification by generating inorganic pyrophosphate (PP_i), a solute that serves as an essential physiologic inhibitor of hydroxyapatite deposition. Most affected children die within the first year of life and factors leading to prolonged survival in some patients are unknown. PP_i and inorganic phosphate have mutually antagonistic roles in tissue mineralization. We hypothesized that hypophosphatemia may balance against pathologic effects of deficient E-NPP1 mediated PP_i generation and may be associated with prolonged survival. We measured serum phosphate and renal tubular phosphate reabsorption (TmP/GFR) levels in 7 surviving patients with GACI older than two years carrying *ENPP1* mutations and in one surviving patient without identified *ENPP1* mutations. Furthermore, in those patients with abnormal phosphate levels, we amplified all 22 *PHEX* and 3 *FGF23* exons by PCR using intronic primers. PCR products were sequenced bidirectionally. 6 of 7 patients with *ENPP1* mutations and the one patient without a mutation had serum phosphate levels below 0.9 mmol/l (normal: 1.38 - 2.01 mmol/l) and TmP/GFR levels below 0.9 mmol/l (normal: 1.45 -2.26 mmol/l). No pathogenic *PHEX* or *FGF23* mutation was found. Hypophosphatemia due to renal phosphate wasting is associated with prolonged survival in GACI and may compensate the phenotype. Renal phosphate wasting is not caused by mutations in *PHEX* or *FGF23* in GACI patients.

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Familial thoracic aortic aneurysms/dissections with patent ductus arteriosus: Perhaps a novel locus. *V.T. Tran-Fadulu, J.H. Chen, H. Pannu, A.L. Lafont, D.M. Milewicz.* Internal Medicine, Med Gen, The University of Texas-HSC, Houston, TX.

Thoracic aortic aneurysms and dissections are important causes of sudden death, and are the 13th most common cause of death in the U.S. Familial cases account for 20% of all TAAD cases. Familial thoracic aneurysms and dissections (TAAD) is an autosomal dominant condition with reduced penetrance and variable expression. Four loci predisposing individuals to TAAD have been mapped to 5q13-14 (TAAD1), 3p24-25 (TAAD2), 11q23.2-q24, and most recently 15q (TAAD3). We have identified 290 families with predominately ascending aortic aneurysms leading to type A dissections. Four of these families had a unique presentation involving both type A aortic disease and patent ductus arteriosus (PDA). None of the four families exhibit features of Marfan syndrome, Ehlers-Danlos syndrome, vascular type or Char syndrome. Affected individuals in these families exhibit a predisposition to dissection and aortic enlargement. Aortic dilation in these families can also occur distal to the sinuses of Valsalva. Linkage analyses revealed that the aortic disease in these families was not linked to any of the known loci for familial TAAD. Familial TAAD with PDA has rarely been described in the literature, and is likely to be a heritable genetic disorder, unlinked to any of the known TAAD loci. Of the four families with this unique phenotype, there were a total of 20 affected individuals with either TAA or TAAD. A total of five cases of PDA were observed; three of which were associated with ascending aneurysms or type A dissections. Two cases were observed in individuals who are asymptomatic of TAAD. The large number of individuals with PDA compared to the incidence of 1 in 2000 in the general population, as well as its co-segregation with TAA/TAAD suggests the presence of a rare novel locus, yet to be mapped, is responsible for this unique phenotype.

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Investigation of 7q11.23 microdeletion in supravalvular aortic stenosis. *N. Abdelmoula[1], B. Gargouri[1], I. Trabelsi[2], S. Kammoun[2], A. Amouri[3], T. Rebai[1].* 1) Lab of Histology, university of Medicine, Sfax, Tunisia; 2) Dep pf Cardiology, EPS Hedi Chaker, Sfax, Tunisia; 3) Lab of Cytogenetics, Pasteur Institute, Tunis, Tunisia.

Supravalvular aortic stenosis is a congenital heart disease caused by deletion, mutation or disruption of the elastin gene at 7q11.23. It may occur as an isolated disease or as part of a complex developmental disorder such as Williams Beuren syndrome. A spectrum of elastin gene mutations has been identified in isolated SVAS. 7q11.23 microdeletions have been described in 95 per cent of WBS patients. However, of all WBS individuals proven to be hemizygous for elastin gene, only 50 per cent had documented SVAS. In the present study, we have screened 7q11.23 microdeletion by fluorescence in situ hybridization in eight patients suffering from congenital heart disease and in whom cardiac evaluation confirm the diagnosis of SVAS. FISH studies were carried out using Williams Syndrome Region probe. No chromosomal aberrations was shown by conventional cytogenetic analysis whereas 7q11.23 microdeletion was detected by FISH in three patients (37.5 per cent). Deleted patients were two males and one female aged of 16, 6 and 5 years. WBS was suspected on the basis of dysmorphic facies or neurobehavioral phenotype in 4 patients. Diagnosis was confirmed for only three of them. In the remainder 4 children with isolated SVAS, 7q11.23 microdeletion was absent. Our study confirms the diagnostic usefulness of elastin gene deletion analysis by FISH and provides further evidence that the typical WBS phenotype is associated very often with 7q11.23 deletion. We concluded that screening of 7q11.23 microdeletion must be done for all patients with SVAS because establishment of WBS in the neonatal period and infancy can sometimes be difficult. Detection of the 7q11.23 deletion by FISH is a sensitive confirmatory test and should be performed routinely to facilitate genetic counselling, family screening, and formulation of a long-term management plan for the patient. If no deletion is found in SVAS patients, mutational studies within the elastin gene should be performed to establish a phenotype-genotype correlation.

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Microdeletion 22q11.2 in contruncal heart disease. *G. Bochra[1], NB. Abdelmoula[1], I. Trabelsi[2], R. Smiti[3], S. Kammoun[2], A. Amouri[3], T. Rebai[1].* 1) Lab. of Histology, University of Medicine, Sfax, Tunisia; 2) Dep. of Cardiology, EPS Hedi Chaker, Sfax, Tunisia; 3) Lab. of Cytogenetics, Pasteur institute, Tunis, Tunisia.

Conotruncal heart defects that account for 50-60% of all congenital heart malformations are known to have a strong genetic component and constitute a cardinal component of branchial arch syndromes, such as DiGeorge and velocardiofacial syndromes. 22q11.2 microdeletions associated with the majority of these syndromes, occur in a high percentage of syndromic conotruncal heart defects and those associated with minor extracardiac abnormalities. A possible strategy for early detection of such syndromes is routine screening for 22q11 microdeletion in all patients with conotruncal heart disease. The purpose of this study was to evaluate whether this strategy is preferable to testing on clinical suspicion. At the south of Tunisia, 26 patients with unselected conotruncal heart disease were tested using fluorescence in situ hybridation and two probes LSI DiGeorge N25 (D22S75) region probe N25/ARSA and LSI DiGeorge/VCFS region probe TUPLE1/ARSA. Cardiac phenotypes were:, regular tetralogy of Fallot (9), irregular tetralogy of Fallot (10), double outlet right ventricule (3), transposition of great arteries with interrupted aortic arch type B (1), pulmonary atresia with ventricular septal defect with malalignment of the conal septum (2), pulmonary valve atresia (2). One deletion was identified in a 1.5-years old boy who have a regular tetralogy of Fallot associated with only some dysmorphic features. Based on this study, only 3.8 per cent of conotruncal heart diseases have 22q11 microdeletion. This prevalence is very low, in comparison to previous studies. We conclude that routine FISH testing for 22q11 microdeletion in conotruncal heart disease should be done in newborn infants with conotruncal defect or associated at least with one additional manifestation of DiGeorge or velocardiofacial syndrome phenotype.

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Normal cardiac anatomy in an infant with trisomy 18 and trisomy X (48,XXX,+18). *J.C. Shores[1], G.L. Barnett[2], C.W. Yu[2], C.A. Friedrich[2].* 1) Pediatric Cardiology,; 2) Medical Genetics, Univ. Mississippi Medical Ctr., Jackson, MS.

An infant with dysmorphic features was found to have a karyotype of 48,XXX,+18 in 21 of 21 blood cells. She was born at term via C-section after failing induction. Examination revealed length and weight less than the 10th percentile for term, a large, open fontanelle extending to the level of her eyebrows, a narrow bi-temporal diameter, small, lowset ears with crumpled pinnae, widely-spaced nipples, small palpebral fissures, an asymmetric mouth, overlapping fingers, contractures of the hips and digits, and adduction of the feet with intact passive range of motion. Cranial ultrasound revealed a 3 mm choroids plexus cyst. Ophthalmologic exam revealed non-specific retinal pigmentary retinopathy. Renal ultrasound showed Grade 1 hydronephrosis. Echocardiogram revealed a small PDA, no VSD or ASD and no valvular abnormalities. She was seen again at 4 months and 7 months, when she was not yet crawling and had trouble swallowing baby food. No sleep apnea has been noted. This patient is apparently unique in that she has trisomy for both chromosome 18 and the X chromosome. She also does not have many typical manifestations of trisomy 18. The prenatal sex ratio is 0.9 although in live born infants the sex ratio is 0.63, suggesting strong prenatal selection against trisomy 18 males. It is unknown whether the additional X chromosome has any effect on the severity of findings expected with trisomy 18. Several studies (Vanpraagh et al, 1989; Musewe et al, 1990; Balderston et al, 1990) have shown up to 90% of trisomy 18 patients have unusual congenital heart defects including double outlet right ventricle and polyvalvular disease. Again, it is unknown what effect trisomy X has on the expression of trisomy 18. Cytogenetic study of a second tissue (e.g., fibroblasts) has been offered to the patients mother to evaluate the possibility of X chromosome mosaicism.

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Familial thoracic aortic aneurysms and dissections: Clinical complications, response to interventions, and survival. *J.H. Chen[1], V.T. Fadulu[1], M. Willing[3], D. Aguilar[1], T. Bryson[1], B. Neichoy[1], A. Estrera[2], S. Safi[2], C. Anh[1], D.M. Milewicz[1].* 1) Int Med, The University of Texas-HSC, Houston, TX; 2) Cardiothor Vasc Surg, The University of Texas-HSC, Houston, TX; 3) Ped, The University of Iowa Hospitals and Clinics, Iowa City, Iowa.

Familial thoracic aortic aneurysms leading to type A or B dissections (TAAD) is inherited in an autosomal dominant manner with decreased penetrance and variable expression. Although there have been three loci mapped for the condition, there has been no comprehensive analysis of the phenotype of families with familial TAAD. A cohort of 155 families with 2+ members with TAAD was assessed for the cardiovascular features, inheritance and survival rates. The cohort of 4941 people were recruited since 1992. At risk individuals were urged to get aortic imaging. 811 family members scored as affected based on the presence of ascending/descending aortic disease. Visual inspection of pedigrees confirmed autosomal dominant inheritance in 49.7% of families. More males were affected (65.8%), suggesting that decreased penetrance is more apparent in women. 90.6% of deaths were due to TAAD. Survival rate in men was 51.7 years (SD=17.7), and in women 53.5 (SD=19.8). 42.1% of affected patients presented with death due to aortic disease. 71 affected people presented with dissection managed medically, and the remaining 399 individuals presented with aortic dilation; with 50% of these detected with dilation after entry into our study and subsequent imaging. Variable expression of TAAD was evident by the age at aortic dissection ranging from age 13-87 years and aortic dilation ranging from 2-85 years. 55.5% of families had vascular disease extending beyond the ascending aorta. Abdominal aortic aneurysms and peripheral vascular aneurysms/dissections were more prominent in men, while cerebral and carotid aneurysms/dissection were more prominent in women. 47 families in the cohort (30.3%) had 1+ members with cardiac abnormalities such as BAV, PDA, and cardiac septal defects. The potential influence of pregnancy on presentation of disease was evaluated. Average time from last parturition to presentation for all women was greater than 20 years.

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Fetal Hypoplastic Left Heart Sequence (HLHS) extracardiac and CNS abnormalities. *M. Thompson[1], A. Many[1,2], S. Keating[1], E. Jaeggi[4], J. Smallhorn[4], D. Chitayat[2,3].* 1) Dept. of Pathology and Lababoratory Medicine &; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital; 3) Division of Clinical and Metabolic Genetics &; 4) Division of Cardiology, Dept. of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Objective: To provide information on the incidence and type of extracardiac abnormalities in general and CNS abnormalities in particular detected in fetuses with HLHS. Methods: Cases, prenatally diagnosed with HLHS between 1995-2005 were evaluated. Most cases were assessed by both detailed fetal ultrasound and echocardiography. Results: Of 63 cases with HLHS detected prenatally, 31 cases had a thorough autopsy at our institution. Of the 31 autopsies, 30 pregnancies were terminated and one had an IUFD. Of the 31 autopsies, 6 had chromosome abnormalities and in 3 the analysis failed (2 had isolated HLHS and one had extracardiac, non-CNS abnormalities). Of the remaining 22, three had CNS abnormalities: one had Dandy-Walker malformation and other abnormalities, one had absence of olfactory bulbs and tracts, absence corpus callosum, retrocerebellar cyst and ventriculomegaly and one had Dandy-Walker variant and corticoneural migration abnormality. Of the remaining 19, 11 had isolated HLHS and 8 had other non-CNS abnormalities. Conclusion: Extracardiac abnormalities are common in HLHS detected prenatally. CNS abnormalities were detected in 3/25 (12%) of the chromosomally normal fetuses.

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A Novel RASA1 Mutation Associated with Vein of Galen Malformation. *D. Chitayat[1,2], G.H.B. Maegawa[1,2], H. Sroka[1,2], L. Boon[4,5], N. Revencu[4], P. Clapuyt[6], M. Vikkula[4], S. Blaser[3].* 1) Prenatal Diagnosis & Medical Genetics Program, Mount Sinai Hospital &; 2) The Hospital for Sick Children, Department of Pediatrics, Division of Clinical & Metabolic Genetics &; 3) Division of Neuroradiology, University of Toronto, Toronto, Ontario, Canada; 4) Human Molecular Genetics , Christian de Duve Institute, University of Louvain Medical School; 5) Center for Vascular Anomalies &; 6) Department of Radiology, St Luc Hospital, Brussels, Belgium.

Vein of Galen malformations (VOGMs) are rare anomalies of intracranial circulation that constitute 30% of vascular malformations presenting in the pediatric age group. Most cases are sporadic and carry low recurrence risk.We report a case with VOGM associated with a novel mutation in the RASA1 gene. The proband was one of monozygotic twins born at 36-week gestation and diagnosed after delivery with VOGM.The pregnancy was complicated with a mild PIH. Fetal U/S confirmed a monochorionic-diamniotic twin pregnancy. The NT of the affected twin was 1.5 mm giving a risk of 1:4,109 for Down syndrome. The proband was born flat and pale and developed tachycardia with poor pulses. She had systolic cardiac murmur 3/6 and CHF.Brain MRI showed a large VOGM, right frontal lobe hypoplasia and cortical dysplasia. There was also immaturity of left frontal lobe. The baby had also nevus flammeus over the mid-forehead and nasal regions, bulbous nose, prominent tongue, intra-uterine growth retardation. Embolization of VOG was successfully performed and neurological follow-up showed no focal signs and a normal development at 9 months of age. Mutational analysis of the RASA1 gene revealed a 5 nucleotide deletion in exon 19, c.2532_2536delTTTAA, causing a frameshift and predicting a premature stop codon.The family history showed that the father had hydrocephalus secondary to Arnold-Chiari malformation.His sister and her two daughters also have facial-capillary malformation.RASA1 mutation was recently found to be associated with capillary and arteriovenous malformation.The finding of VOGM associated with RASA1 mutation add to the spectrum of vascular abnormalities associated with this gene mutation.

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A case of generalizad hypertrichosis asocciated with cardiomegaly in Colombia. *J.C. Prieto[1,2], P.L. Paez[1].* 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Hospital la Victoria, Bogota, Colombia.

A case of a seven year old girl born in Bogotá (Colombia) of nonconsanguineos parents presents chronic pulmonary disease with moderated pulmonary hypertension and left ventricular hypertrophy associated to congenital generalized hypertrichosis. The patient was the product of the mother's thirth term 38-week pregnancy and cesarean delivery because ultrasonographic evidence of polyhydramnios. Birth weight and height was 4000 gr and 50 cm respectively. In neonatal period she had a cyanosis and tachipnea episode then she was admitted at Neonatal Intensive Care Unit where is documented a persistent arteriosus ductus (PAD) without another ultrasonographic finding. She has been hospitalized for multiples episodes of bronchopneumonia and for surgical correction of PAD. Normal psychomotor development. No familiar history. The positive clinical findings are: short stature, evident generalized hypertrichosis, mild bilateral palpebral ptosis, high nasal root, hypoplasic columella, anteverted narines, grade II/VI cardiac systolic murmur, bilateral fifth finger shorteness of hands. Congenital Suprarrenal hyperplasia is discarded. There are not Roentgenologic findings. Hypertrichosis associated with cardiomegaly and osteochondrodysplasia syndrome is characterized for generalized hypertrichosis, cardiomegaly in the vast majority of patients and osteochondrodysplasia more evident at ten years in some patients. Dominant vs recesive heritance mechanism has been described.

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Cantu syndrome in a woman and her two daughters: Further confirmation of autosomal dominant inheritance and review of cardiac manifestations. *D.K. Grange[1], G. Singh[2].* 1) Div. Genetics and Genomic Medicine and; 2) Div. Cardiology, Dept. Pediatrics, St. Louis Children's Hospital, Washington University, St. Louis, MO.

Cantu syndrome, or hypertrichosis-osteodysplasia-cardiomyopathy syndrome, is a rare disorder of unknown etiology. Autosomal recessive inheritance was originally proposed (Cantu, 1982), but a father and his 3 affected children were reported by Lazalde (2000), making autosomal dominant inheritance likely. We report an affected mother and her two daughters, further confirming dominant inheritance. Patient 1 is a 38 year old woman with an enlarged heart since childhood. At age 20 she had a pericardial effusion and underwent pericardial stripping. Cardiac muscle biopsy was normal. She continues to have cardiomegaly and high output cardiac failure with elevated cardiac index on catheterization, but normal LV function. She is tall with macrocephaly, coarse facial features, excessive body hair, large tongue and prominent chin. X-rays show a thick calvarium, wide ribs, platyspondyly and broad metaphyses. Patient 2 is the 15 year old daughter. She has characteristic physical and skeletal findings of Cantu syndrome. She has cardiomegaly and increased pulmonary vascularity. Cardiac mass and chamber sizes are enlarged, but with normal systolic function and no pericardial effusion. Patient 3 is the 9 year old daughter. She also has similar physical features and bony abnormalities. She has marked cardiomegaly and had a pericardial effusion with exercise intolerance and orthopnea, requiring pericardiectomy. Cardiac cath showed enlarged cardiac chambers but normal LV function. 20 previous cases of Cantu have been reported; all had cardiomegaly, but only 2 had pericardial effusion. Cardiac biopsy was normal in one patient (Rosser, 1998) and showed mild disorganization of the muscle in another patient (Nevins, 1996). In our family, cardiac disease has been a prominent feature, with significant pericardial effusions. Ventricular function is normal in our patients, despite enlargement of all chambers and increased cardiac mass, as well as high cardiac output in the mother. Thus, the etiology of the cardiac manifestations in Cantu syndrome remains unclear and warrants further investigation.

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Actual causes of congenital heart defects: a population-based study, Utah 1999-2003. *L.D. Botto, M. Feldkamp, J.C. Carey.* Pediatrics / Div Med Genetics, University of Utah, Salt Lake City, UT.

Heart defects are among the most common birth defects and major contributors to birth defect-associated infant deaths. Finding causes continues to challenge researchers. Although it is said that many cases are due to multifactorial inheritance, this is difficult to prove or disprove. Likewise, epidemiologic studies suggest many weak risk factors, but recognize few definite teratogens, such as retinoic acid or maternal diabetes. We evaluated a population-based cohort of births with major heart defects to identify causes, with a focus on genetic and familial conditions and teratogens. The study cohort included cases occurring among all pregnancy outcomes (livebirths, stillbirths, and pregnancy terminations) from 1999 through 2003 of women residing in Utah, and collected by the Utah Birth Defect Network. We included conotruncal, atrioventricular canal, and left- or right-sided obstructive defects; total anomalous pulmonary venous return; and complex conditions such as single ventricle and heterotaxias. We excluded atrial septal defects, ventricular septal defects, and patent ductus arteriosus. A pediatric cardiologist and clinical geneticists reviewed cases to assess phenotype and causes. We considered familial only instances with a documented affected first-degree relative. We identified 988 cases of heart defects in 239,454 births (rate, 4.1 per 1,000). Clinical review identified a chromosomal condition in 170 cases (17.2% of total), a genetic syndrome in 7 (0.7%), a recognized teratogen in 4 (0.4%) and other etiologies in 5 cases (0.5%). An additional 20 cases (2.0%) were familial using our definition. Thus a known etiology could be ascribed to 206 cases (20.8%). Of the 170 chromosomal cases, 97 (57%)were trisomies. Of the trisomies, 31 (32%) occurred among mother 35 years of age or older. Assuming that maternal age is in the causal pathway, we conclude that a) the main known actual causes of heart defects in this population are maternal age and teratogens; and b) that strict positive family history and known genetic conditions are uncommon causes. These findings underscore the need to aggressively seek causes of heart defects on a population basis.

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Mutations in *CRELD1* are associated with cardiac atrioventricular septal defects in Down syndrome. *C. Maslen[1], D. Babcock[1], L. Bean[2], S. Sherman[2].* 1) Dept Molec/Med Genetics, L465, Oregon Health Sci Univ, Portland, OR; 2) Dept Human Genetics, Emory University School of Medicine, Atlanta, GA.

Complete atrioventricular septal defect (AVSD) is a common and severe form of congenital heart defect. Although sometimes seen as an isolated trait, AVSD is most often found in association with Down syndrome (DS). Trisomy 21 (T21) is not alone sufficient to cause AVSD as only ~25% of DS individuals are affected. However, T21 increases the susceptibility over 2000 fold compared with the general population. We hypothesize that the penetrance of genes associated with isolated AVSD will be increased in the context of T21. We previously identified *CRELD1*, also known as *AVSD2*, as the first known genetic risk factor for AVSD. There is incomplete penetrance, which is consistent with the fact that most cases of non-syndromic AVSD are sporadic. To test our hypothesis, individuals with a DS plus a complete AVSD were analyzed for sequence alterations in the coding region of *CRELD1*. Three different missense mutations were identified in 3 unrelated individuals from a total of 29 cases. All 3 non-synonymous changes are predicted to alter the structure or function of CRELD1. One of the mutations, an arginine to cysteine substitution (R329C), was previously reported in an individual with a sporadic isolated AVSD. R329 is in a calcium binding EGF domain, and the R329C substitution was shown to alter the protein structure as evidenced by a mobility shift on SDS-PAGE. The second mutation results in a cysteine to tryptophan substitution (C221W) in an EGF domain, which will disrupt protein folding. The third mutation, a glutamic acid to lysine substitution (E414K), introduces a change in charge. None of these mutations were identified in 400 race-relevant population control chromosomes, or in 50 chromosomes from individuals with DS and no heart defect. Thus, we have identified 3 mutations in 29 individuals with DS and AVSD (~10%), a higher percentage than that found among isolated AVSD cases (1/42, ~2%). This higher percentage is consistent with our hypothesis that T21 increases the penetrance of AVSD susceptibility genes. *CRELD1* is the first gene to be specifically associated with AVSD in DS.

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The Natural History of Trisomy 12p. *R. Segel[1], I. Peter[2], L. Demmer[1], J. Cowan[1], J. Hoffman[1], D. Bianchi[1].* 1) Dept Pediatrics, Tufts Univ Sch Medicine, Boston, MA; 2) Institute for Clinical Research and Health Policy Studies, Tufts-New England Medical Center, Boston, MA.

Trisomy of the short arm of chromosome 12 is a rare chromosomal anomaly, with an estimated incidence of 1/50,000 births. It may present as a pure trisomy (complete or incomplete), as mosaic trisomy, or as a consequence of a parental translocation, thus involving other chromosomal abnormalities. Prior reports are limited to descriptions of the clinical syndrome at birth or early infancy. Little is known about the natural history or life expectancy. In this study we describe long-term outcome and the differences between patients with mosaic trosomy 12p compared to patients with full trisomy. This study is based on detailed questionnaires to families and physicians, photographs, and medical records. We present a series of 16 patients with trisomy 12p; 6 of them are older than 10 years. Most patients are born at term with normal or above normal birth weight. 7/16 patients were born with a major congenital anomaly, but no single anomaly was present more than once. A clear and consistent dysmorphic facial pattern was apparent in all of the patients. Most patients over 7 years old had a seizure disorder. All patients had developmental delay with speech affected more severely than motor skills. 6/16 patients were described as being very social and interactive. 6/16 had severe behavioral problems and 7/16 had significant sleep disturbances. Facial features of the 3 adult patients were different than the younger individuals. We also differentiated between patients with pure trisomy 12p, mosaic trisomy 12p, and trisomy 12p with other chromosomal abnormalities and showed that the oucome of patients with mosaic trisomy 12p is better than the outcome in the other groups. Patients with trisomy 12p should be followed closely for poor feeding and hypoglycemia in the neonatal period, and for the development of hearing loss, behavioral and sleep problems throughout childhood. This study provides the most comprehensive natural history of trisomy 12p to date, including outcome in 3 adolescents and 3 adults, and demonstrates the clinical variability in this syndrome.

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Late Evening Settling & Early Morning Sleep Maintenance Differentiate the Sleep Patterns of Adolescents & Younger Children with Smith-Magenis Syndrome (SMS). *W.C. Duncan[1], R.S. Morse[2], D. Krasnewich[2], A.C.M. Smith[2].* 1) MAP, NIMH, NIH/HHS, Bethesda, MD; 2) OCD, NHGRI, NIH/HHS, Bethesda, MD.

Sleep disturbance is the major correlate of negative behavior in Smith-Magenis syndrome (SMS). Decreased total sleep begins in infancy and continues through adulthood. However, there have been no formal studies to document possible developmental changes in sleep. Sleep in SMS is often characterized by a phase-advanced profile (early sleep & waking onset). Anecdotal reports suggest that some individuals with SMS have a delayed sleep profile (late sleep & waking onset). In healthy controls, a delayed sleep pattern is often typical of teenagers. This study examines if this delayed sleep pattern is also characteristic of sleep in older children and teenagers with FISH confirmed SMS. We compare in individuals with SMS the nightly activity-rest patterns of subjects > 10 years (n=7) with subjects 10 years (n=15). Additionally, we compare activity-rest patterns of subjects with SMS with an age-matched sibling (SIB) control group (n=10). Activity-rest patterns were assessed by actigraphy, a non-invasive measurement of at-home behavior. Subjects were enrolled in the NIH IRB-approved SMS natural history study and continued on their existing drug regimen. Subjects wore an Actiwatch for 2-4 weeks. Data were analyzed using Actiware-Sleep software (Mini-Mitter Co, Inc.). Activity counts during the first three hours (Early Act) and last three hours (Late Act) were compared, adjusting for unequal variances. Results: Clock times of Sleep Onset ($p=0.17$) and Waking Onset ($p<0.03$) were earlier in younger than in older SMS and SIB groups. Early Act was similar in 10-y SIB and SMS. In contrast, Late Act was increased in 10-y SMS compared with 10-y SIB ($p=0.0034$), and compared with >10-y SMS ($p=0.03$). Early Act was increased in >10-y SMS compared with 10-y SMS ($p=0.04$) and >10-y SIB ($p=0.05$). Preliminary analysis indicated no effect of melatonin on levels of Early and Late Act in children 10-y with SMS. These results indicate treatments for sleep disturbance in SMS should target age-dependent differences in activity levels during early versus late night sleep period.

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Partial trisomy 4q (4q25q31.1) from a familial chromosome insertion with a characteristic mild phenotype. *A. Iglesias[1], M.J. Macera[2], J. Breshin[2], A. Babu[2].* 1) Dept Pediat, Div Gen, Beth Israel Med Ctr, New York, NY; 2) Dept Med, Div Mol Med & Gen, Wyckoff Heights Med Ctr, Brooklyn, NY.

Prenatal diagnosis was done due to history of a familial chromosome rearrangement. The mother had two previous miscarriages, one with a confirmed unbalanced translocation. She is a carrier of a balanced insertion rearrangement between chromosomes 2 and 4; the long arm segment (4q25q31.1) of 4 is inserted in the short arm of 2 at 2p21. Her karyotype was 46,XX,ins(2;4)(p21;q25q31.1).ish der(2)(wcp4+). Amniotic fluid cells analysis in the last pregnancy revealed an unbalanced karyotype consisting of a derivative chromosome 2 from the mother; 46,XY,der(2)ins(2;4)(p21;q25q31.1)mat. The fetal karyotype had a partial trisomy for the long arm segment of 4; 4q25q31.1. The breakpoints designated in both maternal and fetal karyotypes were revised based on comparative genomic hybridization (CGH) performed postnatally using peripheral blood lymphocytes of the proband. After counseling, the couple opted to continue the pregnancy. The proband was born full term. Prenatal and postnatal growths were normal, although head size has been smaller. Motor and language delay were noted early. By 3 ½ years he still had a small head with a narrow forehead. Positive findings were hypertelorism, depressed nasal bridge, inverted v shape upper lip, high palate and vertically placed ears. An asymmetric intergluteal groove towards the left was noted. Hands were short with tapering fingers. Toes were short. Mild muscular hypotonia was noted. By 3 ½ development was appropriate for his age. In summary, we present a case of partial trisomy 4 as a result of a maternal balanced translocation with a distinctive mild phenotype. These findings are similar to previous reported cases confirming the nature of the disorder and suggesting that a trisomy of the distal part of 4q seems to be related with the abnormal development, while the proximal ones are more related with the presence of dysmorphic features. Future cases and the extended use of array-CGH will help to elucidate the genotype-phenotype correlations.

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Familial Cat Eye Syndrome. *D. Waggoner, R. Anderson, S. Schwartz.* Department of Human Genetics, Univ of Chicago, Chicago, IL.

Cat Eye syndrome is a well described clinical condition associated most commonly with an isodicentric bisatellited marker chromosome from 22q. The clinical features can include coloboma, supracuricular ear pits or tags, total anomalous pulmonary venous return, and mild mental retardation. There is significant clinical variability including individuals with mild phenotype. The majority of cases are sporadic but some familial cases have been reported. We report a two generation family segregating a marker chromosome derived from chromosome 22 and show molecular data mapping the breakpoints of the marker. We also review the clinical phenotypes of the familial cases known to date. The proband for our family had total anomalous pulmonary venous return and bilateral preauricular ear pits. Her father was noted to have bilateral preauricular ear pits and lateral vision loss, with normal heart and normal intelligence. A paternal uncle had a unilateral ear pit with normal intelligence. The marker chromosome resulted from breakpoints at 22q11.21 and was found in 100% of the proband cells, was mosaic in the father present in only 45% of cells, and was not present in the paternal uncle. The findings in this family are important in that they illustrate: (1) a rare two generation family reported that emphasizes the variability of clinical features; (2) that familial cases can occur and impact genetic counseling; (3) that parents should be carefully examined for subtle features suggestive of Cat Eye; (4) that although other family members may have subtle features of the syndrome, the marker chromosome may not be present; (5) that although our proband and her fathers breakpoints were in LCR-22-2, there was considerable phenotypic differences between the two individuals and (6) that the mosaicism in the father is most likely due to mitotic loss and may contribute to the differences in phenotype.

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Deletion 13q in an infant with IUGR and dysmorphism - a case report. *K. Yeboa[1], P. Koduru[2], L. Tambini[1], R. Perrone[2], S. Gupta[2].* 1) Medical Genetics, St. Luke's- Roosevelt Hosp. Ctr., NY; 2) Laboratory Medicine, North Shore Univ. Hosp., Manhasset, NY.

Chromosome deletion is generally deleterious due to monosomy for the lost genes. The 13q deletion syndrome is a distinct entity comprising of a number of variable manifestations. Investigations of the part of the lost genome associated with these manifestations have been instrumental in mapping loci for a number of disorders namely, retinoblastoma (RB-1), holoprosencephaly (ZICZ), Hirsch sprung disease (EDNRB) and neural tube defects to 13q33-q34. Here, we present an infant with dysmorphic facie born to a 35yrs old, G3P2, woman with type 11 diabetes. Prenatal history was remarkable for low MSAFP, severe IUGR and oligohydraminos. At 1.2 weeks after birth baby weighed 3.9lbs (10-25%); head was microdolichocephalic with HC:28cm (10%), L:44cm (10%)with narrow bifrontal diameter, small AF. Facial dysmorphism includes hyperteloric eyes, low set ears, depressed nasal bridge; neck was normal, chest symmetrical and abdomen soft. No malformation of cranium, brain, heart and kidneys was noticed. Genitalia was normal, however anus was placed anteriorly. Chromosome analysis showed an abnormal karyotype: 46,XX,del(13)(q31.2q32.3). FISH studies revealed deletion of the subtelomeric region on del(13q) chromosome. Hence karyotype: 46,XX,del(13)(p13q13.1::q32.3q34::q34). Parental chromosomes were normal. Deletion of 13q32 region is associated with major malformations, severe intellectual disability, growth retardation and distal limb abnormalities. Present case has comparatively milder phenotype as compared to the reported cases with deletion of 13q32 region. This may be due to the fact that deletion has not extended to the gene rich 13q32.3 region. Present case is remarkable for growth retardation. Growth hormone deficiency was present in two cases with monosony of 13q31-q32 region. These findings indicate that locus for GH deficiency might reside in this region. We recommend that 1. Chromosome analysis should be performed on all cases of prenatal as well as postnatal growth retardation, 2. All cases with deletion of 13q31-q32 region should be evaluated for growth hormone deficiency.

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The Beckwith-Wiedemann Syndrome: genetic and epigenetic defects in a bipartite cluster of imprinted genes. *R. Della Casa[1], G. Sebastio[1], D. Melis[1], F. Majo[1], R. Tenconi[2], M. Silengo[3], M.M. Rinaldi[4], A. Sparago[5], F. Cerrato[5], A. Riccio[5], Italian BWS Study Group.* 1) Dept of pediatrics, Federico II University, Naples, Naples, Italy; 2) Dept of Medical Genetics, University of Padoa, Padoa; 3) Dept of Pediatrics, University of Turin, Torino; 4) Dept of Medical Genetics, Cardarelli Hospital, Naples; 5) Dept of Environmental Science, SUN, Caserta, Italy.

Alteration of the DNA methylation at the imprinting centres, either IC1 or IC2, and uniparental paternal disomy (UPD) for 11p15.5 alleles are the most common molecular abnormalities in Beckwith-Wiedemann syndrome (BWS). We have characterised the genetic alterations at IC1 and IC2 in a cohort of 56 Italian patients. We detected alteration in IC2 in most of the patients (43%); methylation defects at IC1 and UPD were found in 14% and 18% of the patients, respectively, whereas no mutation was detected in 25% of patients. The table shows the different prevalence of clinical features of BWS as found in the present cohort of patients.

Genotype	Overgrowth	Hepatomegaly	Nephromegaly	Wilms tumor	Omphalocele
IC1	62	50	88	25	0
IC2	50	48	17	0	33
UPD	20	60	40	10	0
No mutation	28	14	28	0	14

The results of this genotype/phenotype correlation suggest that the presence of IC1 alteration is frequently associated with nephromegaly and a higher risk of Wilms tumor. This indicates the need of an accurate surveillance of kidney involvement in BWS with IC1 defect.

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Clinical, ultrasound and histopathological features of girls with ovarian cysts GNAS1 R201 mutated. *L. de Sanctis[1], L. Delmastro[1], L. Artesani[2], A. Linari[3], R. Lala[4], P. Matarazzo[4].* 1) Dept. of Pediatric Sciences, Univ. of Torino, Torino, Torino, Italy; 2) Radiological Service, Regina Margherita Childrens' Hospital; 3) Div. of Pathology, Regina Margherita Childrens' Hospital; 4) Div. of Pediatric Endocrinology,Regina Margherita Childrens' Hospital.

A gain-of-function mutation at codon 201 (R201) in the GNAS1 gene, encoding for Gs-alpha protein, has been described in DNA samples from ovarian cysts in girls with peripheral precocious puberty, isolated or included in the McCune Albright syndrome (MAS). We report the clinical/hormonal, genitopelvic US and ovarian hystological features of 6 patients with ovarian cysts. In all GNAS1 analysis in DNA samples from ovarian cist after cystectomy identified the R201 mutation. All 6 patients began with thelarche or menarche at 4-36 months of age. Cushing syndrome and liver dysfunction was also present in 1 patient, skin dysplasia in 2 others. Hormonal investigations showed peripheral precocious puberty, with low LH/FSH and high 17-beta-estradiol values. At US examination, uterus had adult-like morphology and thin stripes of endometrial echos in all patient, the ovaries were multifollicular with 1-3 monolateral anechogenic cysts, even reaching diameters of 50x42 mm. The macroscopic appearance of the cysts was consistent with the follicular type. Microscopic evaluation showed granulosa cells stratified upon a theca layer; signs of luteinization or presence of smaller cysts in the wall were occasionally recognizable. In a follow-up of 5-8 years, bone and skin dysplasia were disclosed in the patient with Cushing syndrome, and bone dysplasia in the 2 patients with skin dysplasia, thus depicting a classical MAS. Notwithstanding antiestrogen treatment, these 3 patients alternated periods of progression and regression of pubertal development and hyperestrogenism for the appearence of new cysts, even bilaterally. In a follow-up of 5 months-3 years in the 3 subjects with isolated precocious puberty no other MAS signs appeared and pubertal development and hyperestrogenism regressed after cystectomy; in 1 of them an estrogen-secreting cyst recurred monolaterally, responsible for a relapse of precocious puberty.

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Autistic sibships: The role of female gender in determining outcome. *S.K. Martin, J.H. Miles, T.N. Takahashi.* Dept Child Health, Div Med Gen, Univ Missouri Hospital, Columbia, MO.

There is a growing body of evidence that autism (AD) in males and females is phenotypically dissimilar and under different genetic control (Lamb et al., Stone, et al.). We previously reported that females with essential autism have better outcomes, higher IQs and milder AD symptoms than unrelated males (Miles et al. 2004). This study further defines the role of gender and questions whether male-female (MF) sibships are genetically different from male-male (MM). Nine MF and MM sib pairs who met DSM-IV criteria for AD in at least 1 sib and AD or PDD in the other had complete genetics evaluations. MF sibs showed the most disparity. On the ADI-R, sisters had fewer AD symptoms than their brothers in 28 of 36 categories ($p=0.0001$). Sisters outperformed brothers in 13 out of 15 social categories ($p=0.000002$). There were no differences in the areas of communication or repetitive behaviors. Though not reaching significance, sisters had higher mean IQs (100.3 vs 86.5) and were less apt to be macrocephalic (11% vs 56%), factors usually linked to better outcomes. To examine differences between families with and without AD girls, we compared MM and MF boys. On the ADI-R, the MM & MF boys had similar symptom scores overall, though MM boys did better in 12 of the 15 social categories ($p=0.003$); there were no differences in communication or repetitive behavior. IQ scores, handedness, head size and history of regression were no different for boys from MM vs MF sibships. There were no differences in family histories of neuropsychiatric disorders for the MF and MM sibships. This study confirms our previous finding that girls with essential AD are less severely affected than boys. Comparing MM and MF boys revealed no major differences which does not support the model of different genetic control of autism in MM vs MF families. Remarkably, children, regardless of gender, selected both on the basis of essential AD and having an AD sib, had higher IQs, fewer AD symptoms and better outcomes than those from singleton families, indicating essential AD and being an AD sib are additive outcome factors which define a more homogeneous higher functioning autism subgroup.

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LEVEL OF SPEECH: A VARIABLE IN PARENT REPORT OF MOOD RELATED MOVEMENT IN CHILDREN WITH AUTISTIC DISORDER. *R.K. Abramson[1], A.V. Hall[1], S.A. Ravan[1], H. Cope[2], J. Gilbert[2], M.L. Cuccaro[2], H.H. Wright[1], M. Pericak-Vance[2].* 1) Dept Neuropsychiatry, USC School of Medicine, Columbia, SC; 2) Duke University Center for Human Genetics, Durham, NC.

Repetitive behaviors and stereotypies in children with Autistic Disorder (AD) may be influenced by mood. There are few reports of level of speech on parent report of irritability, stereotypy and hyperactivity. **Objective**: To assess the relationship between level of speech, parent report of irritability, stereotypy and hyperactivity on the Aberrant Behavior Checklist (ABC), and Autism Diagnostic Interview-Revised (ADI-R) Factor 1 Repetitive Motor and Stereotyped Behavior (RMSB) (Cuccaro, 2003). **Methods**: Participants (n=81) were drawn from the Duke/USC molecular study of AD. Diagnoses were confirmed by the ADI-R and medical records. The ABC was completed for each individual. Using Discriminant Function Analysis with level of speech (question 19, ADI-R) as the grouping variable, the irritability, stereotypy, and hyperactivity subscale scores from the ABC were combined to create a new factor of mood related movement F-MRM. On this factor, the variables loaded in the following manner, Irritability = 0.697, Stereotypy = -0.270, and Hyperactivity = 0.58. **Results**: Discriminant scores for F-MRM for children with useful speech (n=61) were significantly higher (t=3.042, df=79, p=0.003) than for children with no useful speech (n=20). Pearson Correlation was negative(r= -0.237, p=0.033, n=81) for F-MRM and RMSB, and absent for F-MRM and ADI Factor 2 Insistence on Sameness. **Conclusions**: Useful speech may influence parent report of irritability, stereotypy and hyperactivity as captured by F-MRM. As F-MRM increases, RMSB decreases. Parent report contributes heavily to evaluation of AD and to phenotypic subgrouping for linkage studies. Overall level of speech may be an important variable in parent perception and report of mood related movement.

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Abnormalities of voluntary eye movements in a patient with neurotrypsin defect. *S. Chokron[1], D. Milea[2], F. Molinari[3], A. Consoli[4], D. Cohen[4], A. Munnich[3], L. Colleaux[3], A. Philippe[3].* 1) Fondation Rothschild, Paris, France; 2) Service d'Ophtalmologie, Hôpital de le Pitié-Salpêtrière, Paris; 3) INSERM U393, Hôpital Necker-Enfants Malades, Paris; 4) Service de Pédopsychiatrie, Hôpital de la Pitié-Salpétrière, Paris.

Neurotrypsin mutation is a cause of autosomal recessive mental retardation. To date, three consanguineous families who are all originally from the same area of Eastern Algeria have been identified. We describe the case of a 16 year-old boy in order to define the phenotype associated with neurotrypsin defect. Until he was 18 months old, the first milestones of psychomotor development are normal (age of walking, first words). Clinical examination reveals impaired voluntary saccadic eye-movements in the horizontal and vertical direction whatever the nature of the stimulation (visual, auditory, somesthesic). Eye movements are always associated with compensatory head thrusts. The patient has difficulties to keep visual fixation towards a target. He shows also severe deficit in color discrimination and in face processing. On the other hand, the recognition of animals and objects is correct. Ophthalmological examination is unremarkable. Full scale IQ was 40 on the Wechsler Intelligence Scale for Children (WISC-III) with a verbal IQ score of 46 and a performance IQ score of 46. We analyze these clinical findings with the cerebral MRI results.

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Can pulmonary hypertension in NF1 be secondary to a vasculopathy? *D. Stewart[1], L. Christiansen[2], W. Miller, Jr.[3], W. Nichols[4], M. Pauciulo[4], L. Messiaen[5], G. Tu[6], R. Pyeritz[3], D. Ross[7].* 1) NHGRI; 2) St Joseph's, Reading PA; 3) UPenn; 4) CCHMC; 5) UAB; 6) Lung Center of Nev; 7) UCLA.

Pulmonary hypertension (PH) in neurofibromatosis type 1 (NF1) may be due to pulmonary interstitial fibrosis, a rare complication of the disease. Primary pulmonary hypertension (PPH; not attributable to any known secondary cause) in NF1 has been reported but is poorly characterized. We studied 2 patients with PPH in NF1 with the mosaic pattern of lung attenuation on chest CT, a radiologic finding frequently observed in patients with PH due to vascular disease. We also report the first genetic testing of BMPR2 in individuals with PPH in NF1. BMPR2 is the causative gene of familial PPH. Patient 1. A 72 year-old female with NF1 presented with progressive dyspnea; she had signs of NF1 and cor pulmonale. Chest CT showed a mosaic pattern but no evidence of interstitial lung disease. Right heart catheterization confirmed PH. Evaluation for causes of secondary PH was unrevealing. Mutation testing of BMPR2 revealed a rare, non-conservative SNP in exon 12. Patient 2. A 56 year-old woman with NF1 presented with progressive dyspnea. Chest CT showed a mild mosaic pattern. Cardiac catheterization revealed elevated pulmonary pressures. Evaluation for causes of secondary PH was unremarkable. Her symptoms improved with epoprostenol. No mutations or polymorphisms were identified in BMPR2. Discussion. Pulmonary vasculopathy may underlie PPH in NF1. 1) Vasculopathy in NF 1 is a well-documented manifestation of the disorder described in many vascular beds. 2) Samuels et al (1999) described a patient with NF1 with PH secondary to diffuse intimal thickening from fibrous tissue; the changes were similar to the vaso-occlusive lesions found in the arteries of other NF1 patients. 3) The mosaic pattern observed in our 2 patients can be consistent with a vascular process. 4) The PPH in our 2 patients is not explained by mutations in BMPR2. Conclusion. 1) Vasculopathy of NF1 itself should be considered in a patient with NF1 and PPH. 2) The PPH of NF1 may be responsive to epoprostenol. 3) The role of rare non-conservative SNPs in BMPR2 in patients with NF1 is unclear and needs further exploration.

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***SYMPHONIE* : A knowledge-base to systematically connect symptoms with gene-related diseases.** *M. Ohtsubo[1], S. Moriwaki[1], C.X. Wang[1,2], Y. Hotta[2], T. Horisawa[3], K. Daicho[3], K. Kawaguchi[3], N. Miura[4], N. Mori[5], K. Sato[6], N. Shimizu[7], T. Terao[8], S. Minoshima[1,7].* 1) Photon Med Res Ctr; 2) Dept Ophthalmol, Hamamatsu Univ Sch Med; 3) Chi Co., Ltd; 4) Dept Biochem; 5) Dept Psychiat Neurol; 6) Dept Anat Neurosci, Hamamatsu Univ Sch Med; 7) Dept Mol Biol, Keio Univ Sch Med; 8) President, Hamamatsu Univ Sch Med, Japan.

Symptoms appearing in human gene-related diseases should be invaluable natural phenotypes caused by pathogenic variation in DNA sequence for functional genomics. We have created the words 'symptome' and 'symptomics' by combining symptom with -ome/-omics. In the new paradigm 'Postgenome Symptomics', (a) disease-associated symptoms with genome variation data and various accompanying non-genetic information are widely collected for each of individual cases, (b) they are extensively analyzed and systematically classified/categorized together with reference information from animal models to organize a set of ontologies, and (c) knowledge-bases for various scopes are created with those ontologies as frameworks to integrate collected information. We are carrying out the postgenome symptomics and creating *SYMPHONIE* (SYMPtomics Hamamatsu Ontology for New Investigative Etiology) as a knowledge-base to connect symptoms with gene-related diseases. Various classes of symptom data including chief complaint, physical finding, results of laboratory examination, associating information such as onset age, anamnesis, family history as well as DNA sequence data and disease name if known are being collected for skin and eye diseases as a trial. Current version of *SYMPHONIE* has features such as (1) a diagnosis supporting function by which narrowing-down and search for gene-related diseases using symptom and patient's data; (2) a reference function to obtain the detailed information of each disease and significance for differential diagnosis from similar disorders. The mutation information of disease responsible genes can be seen *via* a hyperlink to *MutationView* which has been constructed at Keio University. *SYMPHONIE* should be useful for clinicians, basic researchers, students as well as pharmacogenomics industries. A prototype of *SYMPHONIE* will be demonstrated.

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Congenital Cataracts, Subcortical Heterotopias, Vermis Hypoplasia , Learning Difficulties and Dysmorphism in a 13 year old Male. *P. Bitoun[1], B. Benzacken[2], E. Pipiras - Pereny[2], J. Gaudelus[3].* 1) Gen Medicine, CHU Paris-Nord, Hosp Jean Verdier, Bondy, Cedex, France; 2) Histo-Embryo-Cytogenetics and Assisted Reproduction Biology; 3) Pediatrics.

Authors present the case of a 13 year old male with a history of congenital cataracts ,facial dysmorphism,learning difficulites mostly with reading and writing cerebellar vermis hypoplasia and paraventricular heterotopias . This young man is followed in visual handicapped clinic since age 6. He was born to a 30 year old G1P1 mother and 29 year old non consanguineous healthy father after an uneventful term pregnancy with meconium fluid and normal apgars. His BW was 3380 grams, Height was 49,5cm , OFC was 34 cm. Bilateral Cataracts were removed by 4 months for lack of visual pursuit.Writing and graphic difficulties were noted at age 4. Evaluation showed midface hypoplasia,short upturned nose,thick lips,cupped ears, short 4th metacarpals,hyperlaxity and he was followed by a speech therapist.A diagnosis of reading and and writing dyspraxia was made. Sensory integration of hearing , visual and kinesthetic stimuli remains difficult. His best corrected distant visual acuity remains at 4/10. He acquired reading and writing but with ongoing difficulties. Fragile X,high resolution caryotype, telomeric screen and GSalpha protein assay were normal, G protein was 91%. Xrays showed pseudoepihysis of the metacarpals. GH ,cortisol,TSH, T3, T4 and PTH 1-84 assay were normal. MRI showed lateral ventricular dilatation; tiny areas of subcortical and paraventricular heterotopias in T2 and flair sequence,pineal gland cyst,vermis hypoplasia and a small pituitary gland. Discussion Periventricular heterotopias have been described by Oda et al (1993), Kamuro and Tnokuchi (1993) and Di Mario 1994 as a possibly X-linked condition but no cerebellar anomalies or cataracts have been reported. This condition appears unique with the association of cataracts and dysmorphism with heterotopias and vermis hypoplasia.

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Angelman Syndrome: a resource to unfold autism genes. *M.T. Bonati[1], F. Monteverdi[1], S. Russo[2], F. Cogliati[2], P. Finelli[2, 3], D. Giardino[2], M. Elia[4], L. Larizza[3].* 1) Clinic of Medical Genetics, Istituto Auxologico Italiano, Milan, Italy; 2) Laboratory of Medical Genetics Istituto Auxologico Italiano, Milan; 3) Department of Biology and Genetics, University of Milan; 4) Neurology Serv., IRCCS Oasi Maria S.S., Troina (EN).

Autism (AD) is a neurodevelopment disorder with a complex genetic etiology. Involvement of 15q11-q13 chromosome region in AD has been indicated by linkage studies and duplications. Moreover, some features found in Agelman Syndrome (AS), such as severe mental retardation, absence of speech, abnormal EEG/epilepsy, inappropriate social behavior, overlap with those often seen in AD. We administered the ADOS protocol 13 AS patients including the known genetic subtypes, namely: deletion of maternally derived 15q11-q13 copy (n=6); paternal chromosome 15 uniparental disomy (UPD)(n=2), imprinting center (IC) defects (n=1) and UBE3A mutations (n=4). The parents were administered the ADI-R protocol. Five out of 6 deleted and one of the two UPD patients received the ADOS classification of autism. One deleted patient and three out of 4 AS with UBE3A mutation fitted the autism spectrum category. The remaining patients were out of the spectrum. There were no differences in autism diagnosis as a result of age. Excluding the IC defects, one may extrapolate that AS patients belonging to each class can receive a comorbid diagnosis of autism and that less severe autistic symptoms seem to privilege patients with UBE3A mutation than those with the deletion. A comparison of social and communication ADOS-items between the AS group and 10 selected idiopathic autism patients was undertaken. Deficits in both domains of the comorbid autism group mirrored those of the idiopathic autism patients, with a quantitative difference, as the following items scored higher/worse in the second group: frequency of vocalization to others, intonation abnormalities, unusual eye contact, social smile, integration of gaze and other behaviors during social overtures. Development of the study by testing a higher number of patients will provide highlights on the behavior overlap between AD and AS consistent with a role in AD etiology for UBE3A and neighboring genes.

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Autism and Alcoholism: A quest for genetic linkage. *C.D. Sauer, T.N. Takahashi, J.H. Miles.* Dept Child Health, Div Med Gen, Univ Missouri Hospital, Columbia, MO.

Autism (AD) is a complex neuro-developmental disorder that has a heterogeneous genetic etiology and a wide array of behavioral and phenotypic traits. Our previous family studies demonstrated a significant genetic overlap between autism and alcoholism with 35% of AD children having a significant family history of alcoholism (Miles et al., 2003). Autism and alcoholism are both highly heritable and etiologically heterogeneous disorders and for both there is biochemical and clinical support for some shared genetic origins. Dysregulation of the dopamine and serotonin neurotransmitter systems occur in both autism and alcoholism. In addition, there is an overlap in drugs used to treat both disorders. In our families, significant family loading for alcoholism correlated with regressive onset autism (52.5% vs. 35.8%, p= 0.04) and decreased incidence of macrocephaly in the child with autism (14.7% vs. 40.6%, p=0.0006). This suggests that the overlap with alcoholism may hold for a subset of autism and that there may be genes common to both disorders. We reviewed published autism (8) and alcoholism (4) linkage and association studies to determine areas of possible genetic overlap. Regions were considered positive for overlap if the linkage peaks were no more than 10 cM apart. Four areas of overlap were identified on chromosomes 2q11, 4q21-23, 15q21 and 16p13. There are a number of evocative genes in these shared regions. Region 2p11.1-q12.2 contains gene BAFME2, associated with various forms of epilepsy. The mutually linked 15q21region contains a dyslexia susceptibility locus, DLX1 and region 16p13 contains the EIM gene for infantile myoclonic epilepsy. The significance of the gamma subunit of alcohol dehydrogenase gene at 4q21-23 is unclear since alcohol dehydrogenase has no apparent relation to autism. Genetic linkage and association studies of complex behavioral disorders have been hampered by poor replicability. We suggest that molecular study of an appropriately chosen subgroup of families selected on the basis of both autism and alcoholism may improve the detection of linkage for these complex neurologic disorders.

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Clinical and morphological characterization of Lathosterolosis. *G. Andria[1], M. Rossi[1], I. Parisi[1], R. Tuzzi[1], A. Battagliese[1], A. Pepe[1], R. Vecchione[2], M. D'Armiento[2], M. Cervasio[2], L. Terracciano[2], G. Corso[3], P. Ferrari[4], F. Rivasi[5], G. Parenti[1].* 1) Dept of Pediatrics, Federico II University, Naples; 2) Dept of Pathology, Federico II University, Naples; 3) Dept of Clinical Biochemistry, University of Foggia; 4) Dept of Pediatrics, University of Modena; 5) Dept of Pathology, University of Modena, Italy.

Lathosterolosis (LS) is a rare defect of cholesterol biosynthesis (DCB), due to the deficiency of 3-beta-hydroxysteroid-delta-5-desaturase (SC5D). Only two patients have been reported in the literature, both with multiple congenital anomalies and liver involvement (cholestasis in both and massive mucolipidosis-like inclusions in one). Here we report on the characterization of a third case of LS, suggesting intrafamilial variability of LS and expanding the phenotypic spectrum of the disease. The patient was an aborted fetus, sibling of the first case described by us, presenting with four limbs hexadactyly, clubfeet and lumbosacral meningocele. The diagnosis of LS was made retrospectively by molecular analysis of the SC5D gene in DNA samples obtained from histological preparations from the fetus. The fetus carried the same two mutations as her living sister (R29Q; G211D). The fetal phenotype suggests a possible involvement of cholesterol biosynthesis derangement in neural tube closure. Liver histology by optic microscopy showed normal portal tracts, marked extramedullary hematopoiesis with atrophy of hepatocytic laminae and hemosiderinic pigment within periportal hepatocytes and Kupffer cells. These histological features have been previously reported as signs of pre-natal liver disease in other DCB. No foamy histiocytes were noted. We also looked for the presence of inclusions in skin fibroblasts from our living LS case. Fibroblasts were cultured in delipidated medium for 3-7 days and examined by electron microscopy. Lamellar inclusions, similar to those found in the other previously reported LS case, were noted, confirming that this is a feature of LS.

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Robinow Syndrome without mesomelic shortening of limbs. *O.A.P. Artigalás, L.O. Dewes, M.B. Golbert, F. Hauser, M.T.V. Sanseverino, J.C.L. Leite.* Medical Genetics Service, Hosp Clin P Alegre, Porto Alegre, RS, Brazil.

This report describes a female newborn, born after a 41-week pregnancy. It was the couple's first child, without consanguinity or family history of malformations. The mother was 34 years old, with an uneventful pregnancy. The mother denied use of any medication, drug or alcohol during regnancy. The first ultrasonography was performed in the 11th week of pregnancy, and it showed an increased nuchal translucence (4,9 cm). The mother was referred for evaluation at our service, where some tests where performed: a normal fetal ecocardiography, an amniocentesis resulting in a 46,XX cariotype, negative tests for infecions, and a color ecodoppler showing a single umbilical artery and a protodiastolic incisure in both uterine arteries. The birth was by vaginal delivery, weight of 3845 grams, lenght of 50 cm and head circunference of 35cm. Apgar score was 9 at 1st minute. Full skeletal x-rays and cranial CT scan showed no abnormalities. The physical examination showed bilateral enlarged thumbs, bilateral bifid hallux, enlarged eyelid slits, macrostomia with gingival hypertrophy and ankiloglossia, auricular pits, bilateral duplicated earlobes, normal female genitalia, pre-sacral pit. These findings were considered consistent with the diagnosis of Robinow syndrome. The syndrome is characterized by a face that reminds of a "fetus", with proeminent forehead, hypertelorism, enlarged mouth, small nose with anteverted nostrils. There may be an important gingival hypertrophy. Other findings include micropenis,hydronephrosis or urinary tract infections, cleft lip an palate, bifid fingers. Congenital heart malformations have been described in some cases. About 80% of the cases present with mesomelic shortening of limbs. Families with autossomal recessive and dominant cases have been reported. The recessive families seem to have more severe anomalies of the vertebrae and mesomelic shortening of upper limbs. In the recessive families, there are homozigous mutation in the ROR2 gene, located in the 9q22 cromossome. The gene for the dominant type is not known yet. The patient we present is being evaluated for ROR2 and possible candidate genes for the dominant type.

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Evaluation of Limb Phenotypes in Patients with Cornelia de Lange Syndrome. *M.A. Deardorff[1], L.G. Jackson[1, 2], D. Yaeger[1], I.D. Krantz[1].* 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Obstetrics and Gynecology, Drexel University School of Medicine, Philadelphia, PA.

The Cornelia de Lange syndrome (CdLS) is a multisystem developmental disorder with facial dysmorphia, hirsutism, cardiac defects, growth and cognitive retardation, gastrointestinal abnormalities and limb malformations primarily of the upper limbs are primarily affected that range from relatively normal small hands to oligodactyly and severe reductions of the entire forearm. Lower limbs are generally spared but may have syndactyly of toes 2 and 3.

Mutations in NIPBL, the human homolog of the Drosophila melanogaster Nipped-B gene, have been shown to cause CdLS. Currently, we are able to detect mutations in approximately 45% of CdLS probands. While frameshift, nonsense, splice site and missense mutations have all been identified, the majority of mutations are protein truncating and likely result in haploinsuffiency as a disease causing mechanism.

To characterize mutations in NIPBL and limb deformities in CdLS, we performed a retrospective analysis of records for 840 patients with CdLS. Both severity of reduction and laterality of defect were scored. Overall, 17% had severe reductions, 15% had moderate reductions and 67% had milder deformities. Mutation positive rates for these subsets were 74%, 57% and 28% respectively. Truncating mutations were seen in 93% of patients with severe reductions and 57% in both the moderate and milder classes. Of those with reductions, 44% were more severe on the right, 19% were more severe on the left and 37% were symmetric.

Our data suggests that patients with more severe limb reductions are more likely to have identifiable mutations compared with those with less affected limbs. This raises further questions about the sensitivity of limb development to the dosage of NIBPL. Furthermore, the finding that the right limb is more often affected suggests that NIPBL may interact or be influenced by mechanisms that determine left-right laterality.

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A patient with acromesomelic dysplasia-type Maroteaux associated with abdominal pseudo aortic aneurysm. *H. Ohashi[1], Y. Ohashi[1], H. Mochizuki[1], N. Mitsui[1], M. Saitoh[2], M. Sakuragi[2], G. Nishimura[3].* 1) Saitama Children's Medical Ctr, Saitama, Japan; 2) Saitama Cardiovascular and Respiratory Center; 3) Tokyo Metoropolitan Kiyose Children's Hospital.

Acromesomelic dysplasia, type Maroteaux, (AMDM) is a rare autosomal recessive skeletal dysplasia characterized by severe dwarfism with shortening of the middle and distal segments of the extremities. Both missense and protein-truncating mutations in natriuretic peptide receptor 2 gene (NPR2) have recently been reported to cause AMDM (Cynthia et al, 2004). We report here a 28-year-old Japanese male with AMDM associated with abdominal pseudo aortic aneurysm. The patient was born to healthy but consanguineous parents (first cousin). His father is 155 cm (-2.75 SD) and mother 153 cm (-0.98 SD) in height, respectively. The patient was initially diagnosed in infancy as achondroplasia because of short limb dwarfism and relative macrocephaly. His mental development was normal. At age 27 years, he suffered from severe abdominal pain and was found to have abdominal pseudo aortic aneurysm, which was subsequently treated with a stent graft. When seen by us at age 28 years, his height was 118.5 cm (-9.3 SD), OFC 57.1 cm (+0.92 SD). His features noted were long face, hypertelorism, marked shortening of the middle and distal limbs, relatively large big toes, and redundant skin of fingers. X-ray study revealed marked shortening of long bones of forearms and hands, and mild platyspondyly. Based on these findings, we concluded that he has AMDM. Sequencing analysis of entire coding region of NPR2 gene showed that the patient has homozygous 1972C>T (L658F) mutation, and his parents are heterozygous carriers. To our knowledge, aneurysm found in our patient is a previously unreported complication in AMDM. We considered two following possibilities: 1) aneurysm is a coincidental occurrence in the patient, and 2) aneurysm is a rare but important complication of AMDM especially in adulthood.

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Craniosynostosis-Marfan-like syndrome with normal intelligence and apparently increased risk of aortic dissection in 6 patients - from Furlong to Loeys-Dietz syndrome. *S.O. Lewin[1], J.M. Opitz[1], J.C. Palumbos[1], K.M. Dent[1], P. Lenglet[1], P. Bayrak-Toydemir[3], H. Pannu[2], A. Lafont[2], D.M. Milewicz[2], J.C. Carey[1].* 1) Div Medical Genetics, 2C412SOM, Univ Utah Medical Ctr, Salt Lake City, UT; 2) Univ Texas Medical Sch Dept Internal Medicine 6431 Fannin, MSB 4 202 Houston TX 77030; 3) ARUP Laboratories Dept of Pathology 500 Chipeta Way Salt Lake City UT 84108.

We present 6 patients to delineate further a craniosynostosis-Marfan-like condition suggestive of Shprintzen-Goldberg syndrome (SGS)with normal intelligence and an apparently higher risk for aortic dissection at a young age. The recent paper by Loeys et al (*Nature Genetics*, March 2005) describing mutations in TGF1 and 2 receptors in 10 families with findings that overlap but are not typical of Marfan syndrome or SGS has confirmed a clinical impression we have had for several years that some patients called SGS have a disorder we called Furlong syndrome (Furlong et al., 1987). Findings in our 6 patients (3 male) included: craniosynostosis, mild hypertelorism, marfanoid habitus, arachnodactyly, eye involvement (esotropia, orbital hypoplasia) no lens dislocation in all. Intelligence was normal in all. Three patients had fatal aortic dissection at 4.5, 12 and 16 years respectively. All had been followed on a Marfan protocol. The finding of mutations in TGFR1 and 2 in the patients of Loeys et al spurred us to look in our patients. Of the 3 patients who died, one has a confirmed mutation in TGFR2, one has no mutation identified in TGFR1 or 2 and the third was not studied. The remaining 3 patients are still being being tested. The pathogenesis of the craniosynostosis-Marfan-like disorders is still unclear, and the role of TGF receptor family and its interaction with fibrillins is being explored. There is an urgent need for clinicians to distinguish which patients may have an increased risk of aortic and other vascular rupture at a young age so they can be followed closely. Patients with atypical Marfan or SGS should have TGFR1&2 genes studied as part of their diagnostic workup.

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The deletion 7q36.3 syndrome. *J. Pappas, E. Moran.* Human Genetics Program, New York Univ, Sch Medicine, New York, NY.

We present three unrelated cases with deletions in the chromosome band 7q36. Case 1 and 2 have terminal deletions that encompass the band 7q36.3 and case 3 has interstitial deletion 7(q36.1q36.3). The presentation of case 1 and 2 was similar to ten published cases with deletion 7q36.3 and included microcephaly, growth retardation with gastroesophageal reflux, mild to moderate mental retardation, hypertelorism, ptosis or blepharophimosis, midface hypoplasia, malar hypoplasia, absent incisors, midline cleft palate and broad tip of the nose. Case 3 presented with developmental delay without any dysmorphic features or malformations. The del(7)(q36.1q36.3) in case 3 was established with chromosome analysis of peripheral blood using G-banding. FISH using subtelomeric DNA probes (Vysis, Inc) was normal indicating that the deletion was not including the D7S427. The initial karyotypes of cases 1 and 2 were reported normal. Subtelomeric FISH revealed deletion of D7S427 in cases 1 and 2 and the deletions were subsequently characterized by G-banding to be del(7)(q36.2) in case 1 and 46,XY,der(7)t(7:8)(q36;q24.3) in case 2. The duplication 8q24.3 contributed minimally to the phenotype of case 2 (Pappas, J ACMG meeting 2004). Genes localized in 7q36.3 include SHH and HLXB9 that are respectively associated with holoprosencephaly spectrum and the Currarino syndrome. Horn, D et al (2004) suggested that patients with deletion of 7q36.3 present with minimal clinical expression of these syndromes. We suggest that case 1 and 2 and the ten published cases with similar clinical and cytogenetic findings have the deletion 7q36.3 syndrome. Four out of these twelve cases have hypoplasia of the corpus callosum that can be associated with haploinsufficiency of the SHH gene. Five out of these twelve cases have sacral abnormalities that can be associated with haploinsufficiency of the HLXB9 gene. Our cases provide further support that the deletion 7q36.3 syndrome is a distinct contiguous gene syndrome with specific dysmorphic features. We suggest that the critical area of the syndrome is 7q36.3 encompassing the SHH and HLXB9 genes and the marker D7S427.

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WAGR caused by a novel unbalanced t(11;15)(p13;p12) demonstrating a 7 megabase deletion by fluorescent in situ hybridization. *P.A. Lennon[1], D.A. Scott[1], A. Eglash[2], D.S. Wargowski[3], A. Patel[1], S.W. Cheung[1].* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Family Practice, University of Wisconsin, Mt. Horeb, WI; 3) Department of Pediatrics, University of Wisconsin-Madison School of Medicine, Madison, WI.

Aniridia usually occurs in isolation, but may also occur as part of the WAGR contiguous gene deletion syndrome, which includes Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation. Variability in WAGR phenotypes led to the molecular characterization of the breakpoints on 11p13 involved in the deletions associated with the WAGR syndrome. This helped to identify the gene positions of PAX6, which predisposes for aniridia, and of WT1, which predisposes for Wilms tumor. We present a female infant with aniridia, bilateral posterior capsular cataracts, nystagmus, left-sided glaucoma, mild unilateral hydronephrosis, and gross motor delay. Family history was negative of relatives with aniridia or similar eye abnormalities. Chromosome analysis revealed an unbalanced t(11;15)(p13;p12). Subsequent WAGR fluorescent in situ hybridization (FISH) probe panel analysis demonstrated deletion of all four clinical probes, verifying haploinsufficiency for both PAX6 and WT1. FISH-mapping of the breakpoints on chromosome 11 revealed a 7 Mb deletion within 11p1311p14. This combination of clinical and cytogenetic findings strongly suggests a diagnosis of WAGR. Genotype-phenotype comparisons between this patient and other reported patients with deletions and/or translocations involving the region between 11p1211p14 will be presented, including patients with WAGR and WAGR + obesity (WAGRO).

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Fetal Hypoplastic Left Heart Sequence (HLHS) and Chromosomal Anomalies. *A. Many[1,2], M. Thompson[1], E.J.T. Winsor[1], S. Keating[1], E. Jaeggi[4], J. Smallhorn[4], D. Chitayat[3,5].* 1) Dept. of Pathology and Laboratory Medicine; 2) Dep. of Obstetrics & Gynecology; 3) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital,; 4) Div. of Cardiology; 5) Div. of Clinical and Metabolic Genetics,Dept. of Paed., The Hospital for Sick Children, Univerisity of Toronto, Toronto, Ontario, Canada.

Objective: To provide information on the incidence and type of chromosome abnormalities detected in cases detected prenatally with HLHS. Methods: Prenatally diagnosed cases with HLHS between 1995-2005 were evaluated. Most cases were detected by both detailed fetal ultrasound and echocardiography. Results: 63 pregnant women with fetal HLHS were identified and chromosome analysis was done in 47. The karyotype was abnormal in 11 (17%). In 3 cases, trisomy 13 was detected and other chromosomal abnormalities were also observed such as trisomy 18 (1 case), 46,XX, der(6)t(6;18)(p24;q22)mat (1 case), 47,XX,+21/46,XX (1), 47,XX,+21/46,XX (1), 45,X (1 case), 45,X/47,XXX (1 case), 47, XX i(12)(p10)/46,XX (1 case), 46,XY. ish del(X)(q26.2q26.2)(Z1C3-)mat (1 case) and 46,XX,del(22)(q11.2q11.2)(TUPLE 1-) (1 case). Conclusions: chromosome abnormalities are common in prenatally detected HLHS. All chromosomally abnormal cases had additional extra cardiac abnormalities and one of the cases had deletion at 22q11.2.

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Subtelomeric Familial Rearrangement Between the Telomeres of 7p and 20q Resulting in Partial Trisomy 20qter/Partial Monosomy 7pter: Clinical Phenotype and Molecular Cytogenetic Analysis. *M. Al-Owain[1], M. Iqbal[2], N. Sakati[3], Z. Al-Hassnan[1], A. Teebi[4].* 1) Department of Medical Genetics, King Faisal Specialist Hospital and Reserarch Center, Saudi Arabia; 2) Department of Pathology, King Faisal Specialist Hospital and Reserarch Center, Saudi Arabia; 3) Department of Pediatrics, King Faisal Specialist Hospital and Reserarch Center, Saudi Arabia; 4) Division of Clinical and Metabolic Genetics, the Hospital for Sick Children, Toronto, Canada.

We report 5-½ year old Saudi girl with dysmorphic facial features, growth retardation, complex congenital heart disease, and anorectal anomalies. Her weight is 11.6 Kg (<<5%), height is 103 cm (5%), and OFC 43.75 (<<2%). She has peculiar facial features with small triangular face, prominent and posteriorly rotated ears, high nasal bridge, thin lips, and high-arched palate with palatine ridge; and small hands and feet. The cardiac malformation consists of double outlet right ventricle, large ventricular septal defect, mild subvalvular pulmonary stenosis, and malposition of great arteries. She had imperforate anus with rectovestibular fistula. Ultrasound of the kidneys was normal. Skeletal survey demonstrated anterior subluxation at S2-3 level, otherwise normal. MRI of the brain showed pachygyria and polymicrogyria with thin but well-formed corpus callosum. Formal developmental assessment at the age of 5 years revealed significantly immature social and linguistic delays; however her intelligence is entirely normal (IQ 94). Additionally, she has moderate attention deficit and hyperactivity disorder of the combined type, but there is no history of aggressive behavior. Chromosomal analysis revealed normal karyotype. Subtelomeric FISH analysis showed unbalanced translocation between the telomeres of 7p and 20q resulting in partial trisomy 20qter/partial monosomy 7pter. The mother is a balanced carrier of the same subtelomeric rearrangement. To our knowledge this is the first cryptic subtelomeric translocation that involves 7p and 20q.

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Mild to moderate postnatal abnormalities in a child with del(6)(q25.1q25.2). *P. Koduru[1], S. Gupta[1], R. Perrone[1], J. Antonelli[2], P. Krietzer[2], J. Fox[2].* 1) Dept Laboratory Medicine, North Shore Univ Hosp, Manhasset, NY; 2) Department of Pediatrics, Schneider Childrens Hospital, New Hyde Park, NY.

Hemizygosity of the constitutional genome is one of the leading etiologic factors underlying congenital anomalies, post natal physical and/or developmental delay. Severity of abnormalities is correlated with the extent of deletions. These deletions may be frequent leading to a specific syndrome, or unique; in the latter type delineation of an associated syndrome is difficult. Here, we present a case of a 6.5 year-old patient with an interstitial deletion in distal 6q and mild to moderate postnatal abnormalities. The propositus is the 5lb 6 oz product of 36-week gestation delivered by NSVD to a 25 year-old G2P1 mother. Neonatal period was complicated by poor such and swallow requiring NG tube feeding for the first two years. She began walking at 2 years and started talking by about third year. Endocrinological evaluation was sought at 6 years because of short stature. At this time the patient weighed 15.7 kg, (<5%, 50% for 4 year-old), reached a height of 102 cm (about 10%), and had a HC of 49.5 cm (about 10%). Other physical findings were remarkable for a high broad forehead, a short neck, hypermobility of the elbows and digits, and extensive side burns. The patient had a 2/6 systolic murmur loudest at apex. Cardiac evaluation was significant for myxomatous thickening and prolapse of both the mitral and tricuspid valves. The patient was enrolled in a regular second grade class with supplemental speech therapy. Karyotype of PHA stimulated peripheral blood from the patient showed an interstitial deletion in the distal long of chromosome 6, 46,XX,del(6) (q25.1q25.2); parental chromosomal studies were normal. Several cases of interstitial deletion in the long arm of chromosome 6 are reported; the majority of them are mapped to the region between 6q14->q23. Deletions in the distal long arm are infrequent. There is considerable heterogeneity in clinical features of patients with deletions in the distal region. Nevertheless, all patients had mental retardation; other frequently noticed features included developmental delay, growth retardation, and hypotonia.

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Defining the 3p14 microdeletion syndrome. *F. Mari[1], S. Gimelli[2], T. Pramparo[2], C. Pescucci[1], C. Speciale[1], M.A. Mencarelli[1], G. Hayek[3], M. Zappella[3], O. Zuffardi[2], A. Renieri[1].* 1) Molecular Genetics, Med Genet, University of Siena, Siena, Siena, Italy; 2) General Biology and Medical Genetics, University of Pavia, Italy; 3) Child Neuropsychiatry, Azienda Ospedaliera Senese, Italy.

We here report a 26 year-old male patient with mental retardation and dysmorphic features. Facies is characterized by high forehead, epicanthic folds attenuating with the age, broad nasal bridge, bulbous and broad nasal tip with hypoplastic alae nasi, slightly posteriorly angulated ears of normal length with thick helix and hypoplastic lobules. He has a normal head circumference. He also has very small hands and feet and pes planus, contractures of finger joints and camptodactyly of the 5 finger. He is able to walk even if on a broad base and language is absent. A brain MRI, performed at 14 years, showed hyperplasia of corpus callosum. During the examination he shows an aggressive and self-injuring behavior even if parents refer a quite behavior. Standard karyotype was normal. High resolution karyotype revealed the presence of an interstitial deletion: 46,XY,del(3)(p14.3;p14.1). Whole genome array-CGH analysis confirmed a deletion of about 8 Mb of the short arm of chromosome 3. Interstitial deletions of proximal 3p were reported in other 13 cases. All of them were identified by standard karyotype. Some of them are slightly larger deletions totally missing the band p14 and the others partially overlap extending toward the centromere. The one reported here is the smaller reported deletion. Some dysmorphic features like high forehead, epicanthic folds and dysmorphic ears seem to be consistent with a deletion of this region. A wider use of array-CGH may lead to the identification of additional microdeletions, further delineating a specific syndrome. We are therefore searching for other patients with overlapping deletions in order to further define a potential genotype-phenotype correlation.

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Immunologic Abnormalities in Smith-Magenis syndrome (del 17p11.2). *W. Introne[1], A. Jurinka[1], D. Krasnewich[1], F. Candotti[2], A.C.M. Smith[1,3].* 1) OCD/NHGRI/NIH, Bethesda, MD; 2) MGBB/NHGRI/NIH, Bethesda, MD; 3) Georgetown Univ.Med. Ctr., Washington, DC.

Since its first description in 1982, the clinical phenotype of Smith-Magenis syndrome (SMS) has been well characterized, including minor anomalies, developmental delay/MR, behavioral characteristics, and sleep disturbance. In 1996, Greenberg et al. reported decreased serum immunoglobulin levels (IgM) in 3/13 (23%) SMS patients studied. Clinical history information commonly includes chronic otitis media often leading to recurrent ear tube placement, chronic sinusitis, and upper respiratory tract infections. To further evaluate this relationship, a systematic study of serum immunoglobulins IgA, IgG, and IgM was conducted to determine the frequency of immune function abnormalities. Immunoglobulin profiles obtained at the initial visit were examined in 49 individuals (30F/19M) with confirmed SMS diagnosis (del17p11.2). Mean age was 6.8 yr.(range 6 mo.-21 yr.). All individuals were enrolled on an IRB-approved SMS natural history study at NIH (protocol 01-HG-0109). Observed values were compared with published age normative data (Jolliff et al., 1982) and defined as Low (5%tile), Normal (5-95%tile) or High (95%tile). **Results**: Immunoglobulin abnormalities were observed in 24/49 (49%) of our SMS study group. In general, IgA levels clustered in the lower limits of normal, with 13/49 (26.5%) SMS subjects greater than 2 standard deviations below the mean (Low); one subject had a borderline high IgA level (95%tile). Similar findings were observed for IgG (9/49 Low) and IgM (10/48 Low). All three levels were low in one child; 11 children were Low for two of three immunoglobulins. Only 25/49 (51%) had normal IgA, IgM and IgG levels for age. The observed distribution was significantly different than expected (p=.0001). **Summary**: Low immunoglobulin levels were observed more frequently than previously reported in SMS and may contribute to the recurrent infections observed. Longitudinal assessment of immune function is needed to understand the significance of these findings.

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CHD7 (CHARGE) mutation and successful T-cell infusion in a patient with choanal atresia, hypocalcemia, GU reflux, heart defect, and congenital T-cell failure. *E. Lisi[1,2], L. Hoefsloot[3], A. Baas[3], K. van der Donk[3], D. Loeb[1,4], G. Thomas[1,5], J. Winkelstein[1,6], A. Kline[7], J. Hoover-Fong[1,2].* 1) Johns Hopkins Univ, Baltimore, MD; 2) McKusick-Nathans Inst Genet Med, Baltimore, MD; 3) Dept Human Genet, Radboud Univ Nijmegen Med Centre, Nijmegen, The Netherlands; 4) Dept Onc & Ped, Sidney Kimmel Comp Cancer Center; 5) Kennedy Krieger Inst, Baltimore, MD; 6) Dept Immun & Ped; 7) Harvey Inst Hum Genet, GBMC, Baltimore, MD.

This is a 6 month old Caucasian male with unilateral choanal atresia, glottic web, tracheomalacia, perinatal hypocalcemia, grade 5 vesicoureteral reflux, aberrant left subclavian artery, severe T-cell deficiency, and cupped auricle. He had a tracheostomy and G-tube placement with Nissan fundoplication, and later allogenic peripheral blood mononuclear cell infusion at 3 months. Post-infusion course was complicated by graft versus host disease. However, engraftment was successful, as he tolerated a culture-positive pertussis infection and responded to immunizations. Hypocalcemia and T-cell deficiency are consistent with 22q11 deletion syndrome. Such extreme congenital T-cell failure is not common in CHARGE syndrome, though choanal atresia, GU reflux, and ear anomalies are associated (Coloboma, Heart defects, Atresia of choanae, Retarded growth/development, Genital abnormalities, and Ear anomalies/deafness). An aberrant left subclavian artery is atypical for both disorders. Due to poor T cell quality, initial peripheral blood karyotypes failed. FISH for 22q11 deletion syndrome on interphase nuclei was normal. Sequencing fibroblast DNA demonstrated a 6155-6157 CTC>AGA heterozygous mutation in exon 31 of CHD7 (numbering based on GenBank entry NM_01778). The CT>AG is a nonsense mutation, leading to premature termination (S2052X). The C>A is a silent mutation (R2053R). Parental sequence over these nucleotides was normal. This case illustrates a mutation in a newly identified gene for a disorder often on clinical geneticists differential with 22q11 deletion syndrome. Furthermore, this is an example of extreme congenital T-cell deficiency in CHARGE syndrome with the first reported successful treatment by unrelated donor T-cell infusion.

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Optimal features predicting 22q11.2 deletion syndrome in adults with tetralogy of Fallot. *A.S. Bassett[1,2,3], A. Fung[2], G.D. Webb[3,4], M.A. Gatzoulis[3,5], E. Chow[1,2].* 1) Dept of Psychiatry, University of Toronto, Toronto, ON, Canada; 2) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) Division of Cardiology, Dept of Medicine, University Health Network, Toronto, Ontario, Canada; 4) Adult Congenital Heart Center, Univ of Pennsylvania, Philadelphia, USA; 5) Adult Congenital Heart Program, Royal Brompton Hospital, London, UK.

22q11.2 Deletion Syndrome (22qDS) continues to be under-recognized, particularly in adults. Determining an optimal set of features using standard methods could be helpful in clinical settings where it is infeasible to test for the deletion in all individuals, e.g., adults with tetralogy of Fallot (TOF). We are actively screening for features of 22qDS in patients with TOF followed at a congenital cardiac clinic for adults. We systematically assessed adults for the presence of features including: dysmorphic facies, voice abnormalities including hypernasality, learning difficulties or special education, birth defects and hypocalcemia. We detected a 22q11.2 deletion in 31 of 102 adults (49 M, 53 F; mean age = 32.8y, SD = 10.3y) who had FISH using standard clinical probes. We used area under the receiver operating curve (AUC) to determine which feature(s) were best for detection of 22qDS. Our analyses revealed no single feature was adequate as a good (AUC > 0.8) screening test. A combination of global dysmorphic features, learning difficulties and voice abnormalities yielded the highest AUC (0.83, 95 % CI 0.75-0.91). However, including just dysmorphic facies and learning difficulties yielded a similarly high AUC (0.82, 95% CI 0.76-0.89), suggesting these two clinical features may be adequate in selecting individuals at high risk for 22q11.2 deletions. Cardiologists following adults with conotruncal and other cardiac anomalies should actively assess for the presence of dysmorphic facies and learning difficulties in order to identify patients most likely to have 22qDS.

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Identical twins with Klinefelter syndrome (47,XXY) and gender dysphoria. *C.A. Friedrich[1], S.H. Gleason[2], G.L. Barnett[1], C.W. Yu[1].* 1) Dept Prev Med, Medical Gen,; 2) Dept Psychiatry, Univ Mississippi Medical Ctr, Jackson, MS.

A man was evaluated for depression and gender dysphoria. He had been treating himself with estrogen and aldactone obtained without a prescription and wanted to pursue sex-change surgery. Due to his abnormal hormonal profile, blood chromosomes were studied and showed a 47,XXY karyotype. It was recommended he discuss with his identical twin the twins risk for having Klinefelter syndrome. Cytogenetic study on the brother was performed and he also has a 47,XXY karyotype. He is also interested in sex-change surgery. Both brothers also had low levels of free and total testosterone. Both brothers have had major academic problems, reported speech and articulation problems, and psychological problems that are known to occur often in patients with Klinefelter syndrome. Neither man is interested in pursuing a sperm count analysis at present to assess fertility. Both men declined recommended testosterone replacement therapy because it would interfere with sex-change surgery. Identical twins with Klinefelter syndrome have been reported rarely. Gender dysphoria is not known to be a feature of Klinefelter syndrome, and complicates the standard recommended treatment. The occurrence of gender dysphoria in identical twins may represent the expression of another gene or genes that both patients possess.

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Multiple pterygium syndrome in first cousins. *M.T. Greally.* Dept Pediatrics, Saad Specialist Hosp, Al-Khobar, Saudi Arabia.

Multiple pterygium syndrome may be inherited as an autosomal dominant, recessive or X-linked recessive trait. The syndrome is characterized by webbing of the skin of the neck, joint contractures, and craniofacial, skeletal and other anomalies. In the autosomal dominant variant (OMIM # 178110) both severe and mild symptoms have been reported in the same family. The X-linked recessive form (OMIM # 312150) has been described in a small number of patients and is a lethal variant. At least two autosomal recessive variants have been described: Escobar syndrome (OMIM # 265000) and lethal multiple pterygium syndrome (OMIM # 253290). There is considerable overlap of clinical features between all variants of this syndrome although some abnormalities appear to be syndrome-specific. The affected individuals presented here are from three families of first cousins. The parents are second cousins: two brothers and one sister married to two sisters and one brother. The proband is a 6-year-old boy with clinical features of lethal multiple pterygium syndrome. An older sister, who died at one month of age, had joint contractures, lung hypoplasia and a severe cardiac defect. A younger brother had severe lung hypoplasia and joint contractures and died at 3 days of life. In one family of first cousins 14-year-old twin boys are affected. In the other family there are two affected boys. The proband has webbing of the neck, elbow, knee and axillae, downslanting palpebral fissures, ptosis, low-set ears, micrognathia, rocker-bottom feet, camptodactyly, low posterior hairline, hypoplastic genitalia and a crouched stance. He has kyphosis of the upper dorsal spine with fusion of vertebral bodies, increased interpedicular spaces, spina bifida in the sacral region and left-sided diaphragmatic eventration. All affected children have normal intelligence. The features of multiple pterygium syndrome in the proband combine those described in both Escobar syndrome and lethal multiple pterygium syndrome. This family is currently undergoing investigation for possible location of the gene responsible for this condition. It will be interesting to see if multiple pterygium syndrome shows locus heterogeneity or if the different types of this syndrome are all allelic variants.

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Two familial cases of Parry-Romberg Syndrome--evidence of Mandelian inheritance and vascular involvement?

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Parry-Romberg syndrome is characterized as slowly progressive atrophy of the soft tissues of half the face, accompanied by epilepsy, trigeminal neuralgia, and changes in the eyes and hair. To date, no unifying etiology has been identified although many other clinical associations have been reported: localized scleroderma, brain space occupying lesion, infection and trauma. Schievink et al 1996 reported the assocation of intracranial aneurysms and Woolfenden et al., 1998 demonstrated reversible vessel caliber changes on cerebral angiography. Many affected individuals also manifest stroke, stroke-like(TIA) and migraine symptoms. Therefore, vascular component may play very important role in the pathogenesis of this condition. Most cases were sporadic and review of the literature did not support autosomal dominant inheritance (OMIM). I herein report two familial cases with this condition. Family one composed of one affected mother and 2 affected daughters. Mother was diagnosed at 28 years with Reynolds phenomenon and autoimmune diseases. One daughter was diagnosed at 13 with noticeable facial asymmetry. The other one was diagnosed in 20s with minimal facial asymmetry yet severe headache of vascular nature. Both daughters have very similar MRI findings: abnormal cerebral deep white matter with increased T2 signal. Family two composed of a father and daughter with facial asymmetry, malocclusion and misalignment of teeth. Father was 28 and the daughtor was 3 at diagnosis. My two familial cases suggest that Parry-Romberg Syndrome can present as autosomal dominant inheritance at least in a subset of individuals. The first family also suggests that the ascertainment of this diagnosis may not be correct. While most patients with this diagnosis have facial asymmetry; facial asymmetry may not be the only cardinal feature. The association with autoimmune Reynolds phenomenon which has a strong vascular component serves another evidence for vascular disturbance. I hypothesize the gene for Parry-Romberg syndrome may be inherited as an autosomal dominant trait as a predisposition with multifactorial modification as seen in many autoimmune diseases.

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Cardiovascular Malformations in deLange Syndrome. *B. Strauss[1], A. Lin[2], B. Pober[1,3], V. Dalili-Shoaie[1], S. Malik[4], M. Curtis[5], V. Kimonis[1].* 1) Genetics, Boston Children's Hospital, Boston, MA; 2) Genetics & Teratology, MassGeneral Hospital for Children, Boston, MA; 3) Surgery, Children's Hospital, Boston, MA; 4) Cardiology, University of Arkansas Medical Sciences, Little Rock, AR; 5) Genetics, University of Arkansas Medical Sciences, Little Rock, AR.

Cornelia de Lange syndrome (CdLS) is characterized by distinct facial features, growth restriction, limb and GI abnormalities, and developmental problems. Mutations in the gene NIPBL have been identified recently in association with CdLS (Krantz et al., 2004). Large series have reported cardiovascular malformations (CVMs) in 13-25 percent. The largest genotype-phenotype analysis reported that septal defects were most common but did not analyze CVMs in detail. Upon literature review, we observed a variety of CVMs. We also studied patients from our catchment area as well as from the University of Arkansas. Our review focused on the pattern of CVMs in a total of 64 patients. The most common CVMs were atrial and ventricular septal defects (36/64 or 56 percent), followed by pulmonic valve stenosis (7 isolated, 4 with septal defects for 11/64 or 17 percent). Both exceeded the general population frequency of the Baltimore-Washington Infant Study (37.7 and 7.9 percent, respectively). No other CVM class was more common than in the general population: 1 single ventricle, 6 TOF, 2 atrioventricular canal defect, 6 left sided obstruction (2 classic HLHS, 1 variant, 1 mitral atresia, 1 COA, 1 aortic stenosis), 1 PDA, and 1 aorto-pulmonary window. Thus, septal defects and pulmonic stenosis appear to be over-represented in CdLS. However, both over and under-ascertainment were likely. Many septal defects closed spontaneously; others may have been missed because of no imaging or autopsy. Failure to include milder CdLS cases may limit inclusion of less serious CVMs. We are eager to collaborate to pursue genotype-phenotype studies of the NIPBL gene, using well-defined patients with uniform cardiology imaging. We conclude that diverse CVMs are associated with CdLS, though both septal defects and pulmonic stenosis appear to be more common. (A.L. supported by MA Center for Birth Defects Research and Prevention, Massachusetts DPH.).

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A Congenital, Rhizomelic Skeletal Dysplasia Associated With Bilateral Imperforate Irises. *D.L. Skidmore[1], S. Unger[1,2], A. Superti-Furga[2], D. Rootman[3], A. Levin[3], J. Pierre- Louis[1,4], D. Chitayat[1,4].* 1) Division of Clinical Genetics & Metabolics, Hospital for Sick Children, Toronto, ON, Canada; 2) Center for Pediatrics and Dept. of Human Genetics, University of Freiburg, Germany; 3) Dept of Ophthalmology, Hospital for Sick Children, Toronto, Canada; 4) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada.

The combination of a lethal skeletal dysplasia and abnormality of the anterior chamber of the eye was reported once (Akaba et al., 1996), we report a case of osteochondrodysplasia with absent pupils seemly distinct from this case. Our patient was the product of her parents first pregnancy and her parents were non-consanguineous. Prenatally, short limbs were detected on an ultrasound at 34 weeks gestation (but not at 20 weeks gestation). At birth, she was noted to have rhizomelic limbs, severe platyspondyly, delayed maturity of the pubic bones and gracile ribs and long bones. Craniotabes was also present. There was occipitalization of the posterior arch of C1 with narrowing of the spinal column. The spinal cord itself was not impinged on, and the childs neurological examination and an MRI of the spinal cord and brain were unremarkable. The head was microcephalic with proportionate microphthalmia, with an abnormal anterior segment in which iris failed to perforate, causing absence of the pupils. The structures of the posterior segment of the eye appeared normal on ultrasound and MRI, as did the central nervous system. The child is now several months old and thriving and there has been no evidence of respiratory distress. Her skeletal features did not correspond to those of common dysplasia. This case represents a novel association of congenital rhizomelic skeletal dysplasia with an ophthamological anterior segment abnormality, namely an imperforate iris.

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A Patient with Distinctive Eyebrows, Syndactyly of the Feet, Unilateral Hypoplastic Fifth Finger, and Pili Torti: A New Syndrome. *D. Gul.* Dept. of Medical Genetics, GATA Medical School, Ankara, Turkey.

There is a wide variation in the color, distribution and density of eyebrows. Some hereditary variations are of no known significance, but others are associated with other developmental defects, or are parts of a recognized syndrome. In this report an association of a distinctive eyebrow with other abnormalities in a male is presented. The proband was a 20-year-old male, referred to our hospital because of his facial appearance and foot syndactyly. He was born as second one of four children to healthy nonconsanguineous parents. He was morphologically normal during pregnancy and early childhood. Family history was negative. On admission, his height was 190 cm and weight was 82 kg. The mental status of the individual was normal. He had a mildly remarkable facial appearance: telecanthus, mildly narrow palpebral fissures, dysplastic and posteriorly rotated ears. His hair was curly and dark in general, and dense particularly in the occipital area. The eyebrows were interrupted and arched with late thinning, resembling that of Mr. Spock of the movie, Star Trek. His beard was not dense, but growing upward. Microscopic examination of the scalp hairs showed pili torti. Thumbs and little fingers were short, and on the left, there was a dysplastic nail and marked deficiency. Unilateral incomplete cutaneous syndactyly of the 4th and 5th fingers of the left hand was also noted. Right 5th finger had a single flexion line while left 5th finger was normal.Craniography showed impressiones digitatae. Cytogenetic study with high resolution banding revealed a normal 46,XY karyotype. The case presented here is differentiated from the condition named as tricho-odonto-onchyo dermal syndrome reported by Pinheiro et al. [1981], Kabuki syndrome, and fetal hydantoin syndrome, similar to those of cases reported by Berkenstadt et al. 1988, Gross-Kieselstein and Har-Even 1989. The small number of reported cases would suggest that the phenotypic spectrum of this group of disorders has not been fully defined. This case presented here represents a new distinct entity with bilateral eyebrow abnormality accompanied by multiple congenital abnormalities.

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Spondylocostal Dysostosis with urogenital anomalies: Casamassina Morton Nance Syndrome. *J. Montoya, R. Garcia, I. Zarante, F. Suarez.* Inst de Genetica Humana, Univ Javeriana, Bogota Cundinamar, Colombia.

Newborn female, product of fourth pregnancy, 37 years old mother. Caesarean delivery due to placentary insufficiency, the prenatal ultrasound reported mesenteric cyst, and enhanced, nucal translucency. Karyotype by amniocentesis was a normal 46 XX. On medical examination the patient presented a single umbilical artery, mammary hypertelorism, diastasis of cranial sutures, low set ears and overfolding of the helix, short neck with redundant skin, and a right paraumbilical mobile mass of hard consistency. Rhizomelic shortening of upper limbs, asymmetric short thorax and urovaginal fistula. The Echocardiogram showed pulmonary hypertension and permeable oval foramen. Abdominal Ultrasound found hypoplastic kidneys with increased echogenicity, loss of corticomedular differentiation and in pelvic fossa was observed a tubular and serpiginous structure with echogenic content inside suggestive of intestinal liquid. Thorax X-rays, demonstrated asymmetrical costal arches with right hypoplastic ribs and thoracic hemivertebras. The exact aetiology of the abdominal mass was not definitely establish, the abdominal mass reduce by itself without any specific treatment. We believe that the association of a single umbilical artery, urogenital anomalies and spondylothoracic dysplasia suggested that this is a distinct entity of the Jarcho-Levin syndrome (OMIM: 27300) and the case could be a new patient with the Casamassina Morton Nance Syndrome (OMIM: 271520).

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Characterization of a severe Smith-Lemli-Opitz Syndrome Mutation. *L.S. Correa-Cerro, F.D. Porter.* Heritable Disorders Branch, NICHHD/NIH, Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is a common autosomal recessive metabolic disorder in Caucasians. SLOS is due to a deficiency of 7-dehydrocholesterol reductase (DHCR7) activity that results in elevated 7-dehydrocholesterol levels and typically decreased cholesterol levels. The clinical spectrum ranges from mild to severe. Here we report a severely affected SLOS patient who died 12h after birth. Cranial facial features included an asymmetric calvarial vault, cleft palate and micrognathia. External genitalia were ambiguous. Pathological examination of the heart showed dysplastic mitral valve, patent foramen ovale, patent ductus arteriosus, aortic valvular stenosis, and hypoplastic left ventricle. Karyotype was 46,XY, and chromosome 22 deletions were excluded by FISH using the Tuple 1 probe. The patient was a compound heterozygote for DHCR7 mutations IVS8-1G>C and 957A>G (T319A). We analyzed the effect of the T319A mutation on protein stability and subcellular localization using a Flag epitope tag in COS cells. COS cells were transfected with either a mutant DHCR7 expression plasmid (Flag-DHCR7T319A) or an expression plasmid expressing DHCR7 (Flag-DHCR7). After 24h, RNA was extracted and analyzed for gene expression by relative quantitative PCR. Both the Flag-DHCR7 and the Flag-DHCR7T319A were expressed. Western blot analysis of cell lysates from COS cells transfected with a Flag-DHCR7T319A expression construct detected a slight band of the mutant protein under conditions where the control Flag-DHCR7 protein was very well detected. Immunostaining of cells established the endoplasmic reticulum localization of DHCR7 in COS cells transfected with Flag-DHCR7. In contrast, only background fluorescence was observed in COS cells transfected with Flag-DHCR7T319A. These data suggest that the T319A mutation destabilizes DHCR7 and in combination with a null allele, IVS8-1G>C, can result in a severe phenotype.

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A Japanese Michelin tire baby syndrome patient complicated with Lowe syndrome. *M. Hara[1], T. Kondoh[1], S. Oka[2], A. Tanaka[1], O. Shimokawa[3], N. Harada[3], N. Miyake[4], N. Matsumoto[4], H. Moriuchi[1].* 1) Dept of Pediatr, Nagasaki Univ Hosp, Nagasaki, Japan; 2) Dept of Pediatr, Sasebo Kyosai Hosp, Sasebo, Japan; 3) Kyushu Med Sci Nagasaki Lab, Nagasaki, Japan; 4) Dept of Hum Genet, Nagasaki Univ Grad Sch of Biomed Sci, Nagasaki, Japan.

Congenital symmetrical circumferential skin crease is a cardinal manifestation of Michelin tire baby syndrome (MTBS), and associated with additional clinical features. On the other hand, Lowe syndrome, also called oculo-cerebro-renal syndrome of Lowe (OCRL), is an X-linked recessive disorder characterized with cataract, hypotonia, mental retardation, renal tubular dysfunction and rickets, and is caused by mutation of OCRL1 gene located on Xq26.1. We here report a MTBS patient complicated with Lowe syndrome. Patient, a 21-month-old boy, is the second child of nonconsanguineous healthy mother and father aged 34 and 39 years, respectively, when the boy was born. He was born at 39 weeks of gestation with weight and length of 2,804 g (-1.0 SD) and 48.0 cm (-0.9 SD), respectively. He had bilateral congenital cataracts, for which both eye lenses were removed surgically at 1 month of age. He was also noted to have hypotonia, developmental delay, bilateral undescended testes and multiple circumferential skin creases. Magnetic resonance imaging examination revealed severe thickened adipose tissue around extremities and shoulders. His growth has been deteriorated from 6 months of age, and his height and weight were 71.8 cm (-4.2 SD) and 7,475 g (-3.4 SD), respectively at 21 months of age. Thus, although he was diagnosed as MTBS, he was unique to have bilateral cataract and developmental delay. At 10 months of age, pan-aminoaciduria and renal tubular acidosis were detected; therefore, he was compatible with Lowe syndrome. His total DQ is around 30 at 21 months of age. Microarray-based comparative genomic hybridization analysis disclosed no abnormality using subtelomeric and other BAC/PAC clones including OCRL1 region of Xq26.1. This is the first case of MTBS complicated with Lowe syndrome and further examinations will be needed to clarify whether those two syndromes are etiologically related or occurred by chance.

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Genetic heterogeneity of Shprintzen-Goldberg syndrome. *R. Kosaki[1], D. Takahashi[2], T. Udaka[2], M. Matsumoto[3], S. Ibe[4], T. Isobe[5], Y. Tanaka[6], K. Kosaki[2].* 1) Dept of Clin Genet & Mol Med, Natl Children's Med Ctr, Tokyo; 2) Dept of Pediatrics, Keio Univ Sch Med, Tokyo, Japan; 3) Dept of Orthoped Surgery, Keio Univ Sch Med, Tokyo, Japan; 4) Dept Orthoped Surgery, Ibaraki Prefectural Rehabilitation Ctr, Ibaraki, Japan; 5) Dept of Pediatrics, Ibaraki Children'fs Hospital, Ibaraki, Japan; 6) Dept of Pediatrics, Ichikawa General Hosp, Tokyo Dental College, Chiba, Japan.

Shprintzen-Goldberg syndrome (SGS MIM[182212]) is characterized by craniosynostosis and Marfanoid features. To date, two mutations in FBN1 have been reported among SGS patients: C1223Y and P1148A amino acid substitutions. The heterozygous C1223Y substitution occurred de novo and was located at one of the six highly conserved cysteine residues within the 47 repetitive EGF-like domains; this substitution was thus considered pathogenic. On the other hand, the P1148A substitution, which was initially regarded to be pathogenic, was later found to be a polymorphic variant. Hence, C1223Y is the only FBN1 mutation that has been unambiguously associated with the SGS phenotype to date. Recently, Loeys et al. reported six families with TGBFR2 and four families with TGFBR1 [MIM 190181] mutations who presented with a previously undescribed aortic aneurysm syndrome characterized by hypertelorism, bifid uvula and/or cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm, collectively referred to as Loeys-Dietz aortic aneurysm syndrome. Similarity between LDAAS and SGS was discussed in their paper. We document two patients with classic SGS phenotype: one with a heterozygous TGFBR2 mutation (IVS5-2a>g) leading to a 10-amino acid insertion in the kinase domain, and the other with a heterozygous FBN1 mutation (C1221Y) leading to the disruption of a disulfide bond, as in the C1223Y mutation. In addition, the patient with the FBN1 C1221Y mutation was heterozygous for the T315M substitution of TGFBR2, a known functionally relevant polymorphism. We suggest that genetic heterogeneity is present in the pathogenesis of SGS and that TGFBR2 can act either as the causative gene or as a modifier gene in the pathogenesis of SGS.

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X-LINKED RECESSIVE FUSION OF METACARPALS IV- V ASOCIATED WITH CARPAL SYNOSTOSIS.

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INTRODUCTION Metacarpal synostosis is a rare malformation, clinically variable, and causally heterogeneous. It usually affects the 4th and the 5th metacarpal bones, and can be observed as an isolated anomaly on otherwise normal individuals, or associated with other malformation as the Waardenburg-anoftalmy syndrome. In its isolated form, only three families have been reported showing X-linked inheritance. We report a fourth family with five males affected in three generation, confirming the X-linked recessive pattern of this abnormality. In addition, fusion of carpal bones is segregating in this family, which has not been reported before. CLINICAL REPORT The propositus, a nine years old male, was born after 39 weeks of an uneventful pregnancy from healthy and unrelated young parents. By maternal side, there are other four affected males. At birth, height was 51 cm, weight 3050 gr and head circumference 35 cm. Normocephaly and normal facial features. Upper limbs showed bilateral hipoplasy and ulnar deviation of 4th and 5th fingers. X-ray reveled proximal fusion between IV and V metacarpals with brachyphalangia of the 5th finger; other four members of this family showed the same malformation. The propositus and other two individuals showed additionally carpal fusion (trapezius and trapezoid), with ulnar deviation and tenar hipoplasy. CONCLUSSION We report a family carrying an IV-V metacarpal synostosis, with an additional fusion of the carpal bones. This very interesting association of malformations has not been reported previously. Additionally, this large family confirms the recessive X-linked inheritance mode of this abnormality.

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Phenotypic spectrum of CHARGE syndrome in antenatal and postnatal series with *CHD7* gene truncating mutations correlates with expression during human development. *D. Sanlaville[1], H.C. Etchevers[1], M. Clément-Ziza[1], B. Keren[1], S. Audollent[1], V. Abadie[2], D. Bonnet[2], F. Brunelle[2], G. Couly[2], P. Hubert[2], D. Jan[2], Y. Manach[2], D. Sidi[2], A. Munnich[1], M. Vekemans[1], J. Amiel[1], T. Attié-Bitach[1], S. Lyonnet[1].* 1) Dept Genetics and INSERM U393, Hopital Necker Enfants Malades, Paris, France; 2) Dept Pediatrics and Surgery, Hopital Necker Enfants Malades, Paris, France.

The acronym CHARGE, coined by Pagon refers to a non-random cluster of malformations first described by Hall including **C**oloboma, **H**eart malformation, choanal **A**tresia, **R**etardation of growth and / or development, **G**enital anomalies, and **E**ar anomalies, all features being inconstant and non specific. This Multiple Congenital Anomalies - Mental Retardation syndrome is frequent, and prognosis remains poor. Recently, *CHD7* gene mutations have been identified in CHARGE patients, however the function of *CHD7* during development remains unknown. We therefore studied a series of 10 antenatal cases ascertained on pathological examination and compared with 60 postnatal cases in whom the diagnosis was suspected, considering modified Pagon criteria. The findings of *CHD7* mutations in each of the fetuses allowed us to better delineate the full phenotypic spectrum of the developmental anomalies resulting from *CHD7* haploinsufficiency. Conversely, the percentage of mutations in postnatal series did not exceed 50 %. Interestingly, arhinencephaly and semi-circular canal agenesis were two very frequent features that do not belong to diagnostic criteria so far. Moreover, *in situ* hybridization analysis of the *CHD7* gene during early human development emphasized the role of *CHD7* in the development of central nervous system, internal ear, neural crest of pharyngeal arches, and more generally showed a good correlation between specific *CHD7* expression pattern and the developmental anomalies observed in CHARGE syndrome.

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A Japanese patient with Klippel-Trenaunay-Weber syndrome complicated with renal arteriovenous abnormality. *A. Tanaka, T. Kondoh, Y. Nakashima, H. Moriuchi.* Dept of Pediatr, Nagasaki Univ Hosp, Nagasaki, Japan.

Klippel-Trenaunay-Weber (KTW) syndrome is characterized by large cutaneous hemangiomata with hypertrophy of the related bones and soft tissues. Previously, KTW syndrome patients with hematuria due to the rupture of hemangioma in intrapelvic region have been reported, but macrofistulous arteriovenous (AV) communications are very rare. In contrast, Parkes Weber (PW) syndrome is characterized by a cutaneous flush with underlying multiple micro-AV fistulas, in association with soft tissue and skeletal hypertrophy of the affected limb. Whether KTW syndrome and PW syndrome are in the same entity is unknown. We here report a KTW syndrome patient with renal AV abnormality. Patient, a 9-year-old girl, is the second child of nonconsanguineous healthy mother and father aged 33 and 35 years, respectively, when she was born. Hemihypertrophy of right leg was pointed out at birth. The circumference of the right femoral region was 2.0 cm larger than that of the left corresponding region. The length of the right leg was 2.0 cm longer than that of the left. Her right femoral bone was bigger than the left in bone X-P. Echogram of her abdomen and right femoral region showed enlargement of the right kidney and soft tissue hypertrophy of the right leg, respectively. Magnetic resonance imaging (MRI) of her right leg at 5 years of age revealed soft tissue hypertrophy with subcutaneous hamangioma and venous dilatation. Her karyotype was 46,XX. She was diagnosed as KTW syndrome and followed by orthopedists because of her leg hemihypertrophy. At 8 years of age, she developed macrohematuria. Abdominal echogram with color doppler studies, enhanced computed tomography and MRI examinations were indicative of AV malformation or fistula of her right kidney. Recently, RASA1 and VG5Q genes, both of which are located on 5q13.3, were reported to be causative of capillary or AV malformation seen in KTW syndrome or PW syndrome, respectively. Therefore, complication of AV malformation in KTW syndrome may have implications in the etiology and pathogenesis of the two syndromes. Further radiological and genetic studies are needed to clarify the pathogenesis of renal AV malformation in our case.

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Pterygium colli, craniofacial dysmorphism, asymmetric chest deformity, short stature, microcephaly and global developmental delay: A new MCA/MR syndrome? *EvaM. Tomiak[1,2], GailE. Graham[1,2].* 1) Division of Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada; 2) University of Ottawa.

Pterygium colli, which can be a consequence of prenatal lymphedema, can occur as an isolated finding. It can also be seen in Klippel-Feil sequence, in association with a variety of partial aneuploidies and aneusomies, and in several common and a few rare single gene disorders. Pterygium colli has been described as a component of several MCA/MR syndromes, where it is seen in combination with trigonecephaly and pectus excavatum (Haspelagh syndrome), microcephaly, hypotonia and growth failure (18p deletion), or characteristic facial features (mosaic trisomy 22; partial trisomy 3q). Pterygium colli and MR have also been reported in association with macrocephaly and hypoplastic inverted nipples (al-Gazali LI et al: Clin Dysm 1996; 5(4):321-7), and with edema, epilepsy and pachygyria of the frontal lobes (Fryns JP et al: J Med Gen 2000; 37:460-2).

We describe a 10 year old boy with pterygium colli, global developmental delay, microcephaly, short stature, asymmetry in chest musculature and nipple hypoplasia, who does not appear to fit any of these previously described conditions. He has an extremely long, narrow face with bitemporal narrowing, malar hypoplasia, and prominent anteverted ears. Additional features include valvular pulmonic stenosis, secundum ASD, hypotonia, delayed bone age and minor interdigital syndactyly. There is no history of seizures, developmental regression or behavior problems. Chromosomal, amino acid and urine organic acid analyses have all been normal, as have hand xrays and abdominal ultrasound.

The possibility of a previously unrecognized autosomal recessive condition is raised by the presence of parental consanguinity. Despite extensive literature searches and informal presentation at international dysmorphology meetings, we have not been able to find a unifying diagnosis. We propose that this constellation of features may represent a new distinctive syndrome and hope, by way of this communication, to identify similar individuals at other centres.

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Initiation and preliminary analysis of National Cohen Syndrome Database. *L. Nye, J. Renner, H. Wang.* DDC Clinic for Special Needs Children, Middlefield, OH.

Cohen syndrome (CS) is a rare autosomal recessive disorder with about 100 cases reported. The disease is characterized by facial dysmorphism, microcephaly, hypotonia, developmental delay, myopia and retinal dystrophy, neutropenia, and truncal obesity. The mutation of COH1 gene on chromosome 8q22 is believed responsible for CS. However, we believe that CS remains underdiagnosed or misdiagnosed in many cases in the United States because of rarity of the disease, pleiotropic phenotype, unavailability of clinical testing, and lack of extensive study. As an effort to improve disease diagnosis and clinical management and to promote more effective CS research, we have recently initiated the National Cohen Syndrome Database (NCSD) through DDC Clinic for Special Needs Children in Middlefield, OH, where most Old Order Amish CS patients receive their medical care and service coordination. The questionnaire was distributed to the families of 58 patients registered in our National Cohen Syndrome Family Center; 26 are Amish or Amish-originated (Amish) and 32 non-Amish Caucasians (non-Amish). Thirty-seven completed questionnaires, 20 from Amish and 17 from non-Amish, have been entered in NCSD with 2 patients excluded, as they do not fit the CS phenotype. Average patient age is 21 years (range from 1.5 to 45), with 41% male and 59% female. Preliminary analysis of NCSD has shown that although typical CS features have been documented in almost all patients, a significant phenotype variation has been observed among ethnic groups, even among affected siblings in the same family. Some clinical features, although not reported as part of CS before, have been observed during routine primary care for our Amish patients, and confirmed in most non-Amish patients, such as an unusual cry soon after birth (63%), superior hearing capacity (53%), and anxiety (71%). Surprisingly, among the non-Amish patients, only one has DNA mutation confirmation, reflecting the lack of basic research on CS in the US. The ongoing expansion of NCSD, with the addition of more patients, clinical data, and genotype determination, will hopefully lay some ground work for further CS study in this country.

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A new panel of microsatellite markers in the PAR1 identifies deletions downstream of *SHOX* in patients with Léri-Weill dischondrosteosis (LWD) and idiopathic short stature(ISS). *S. Benito-Sanz[1], N.S. Thomas[2], C. Huber[3], D. Gorbenko del Blanco[1], M. Aza-Carmona[1], J. Crolla[2], V. Maloney[2], J. Argente[1], A. Campos-Barros[1], V. Cormier-Daire[3], K.E. Heath[1].* 1) Dept. Endocrinology, Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, Madrid, Spain; 2) Wessex Regional Genetics Laboratory, Salisbury District Hospital, UK; 3) Dept. of Genetics and INSERM U393, Hospital Necker Enfants Malades, Paris, France.

Léri-Weill dyschondrosteosis (LWD) is an autosomal dominant skeletal dysplasia characterized by short stature and Madelung deformity. *SHOX* mutations have been associated with LWD, Langer mesomelic dysplasia (LMD) and idiopathic short stature (ISS). *SHOX* is located in the pseudoautosomal region 1 (PAR1) of the X and Y chromosome. Previous studies have identified that 60% of LWD patients have a *SHOX* mutation, the majority of which are deletions, whereas in the remaining 40% no genetic defect has been discovered.

We designed a new panel of microsatellites (n=18) in the PAR1 to screen for deletions in the 5' and 3' regions of *SHOX* in LWD/ISS patients in whom no *SHOX* mutation was detected by microsatellite analysis, dHPLC or DNA sequencing. New microsatellites were identified with the help of the Tandem Repeat Finder program (http://tandem.bu.edu/trf.html). We analyzed a cohort of 80 LWD, 18 ISS patients and 2 with Madelung deformity and normal height.

PAR1 deletions downstream of *SHOX* were identified in 12 LWD patients (15%), 1 ISS patient (5.6%) and a patient with normal height and Madelung deformity. The new panel of markers and SNPs within the region allowed us to finely map all 14 deletions to a region 30-530 kb 3' downstream of *SHOX*. Deletions were of variable size (80-500kb) and all shared a common deleted region of 29kb. The identification of PAR1 deletions not including *SHOX* in LWD/ISS patients suggests the presence of either: 1) a position effect affecting transcriptional control of *SHOX* or, 2) the involvement of a second locus in the pathogenesis of LWD and short stature. (sara_bsanz@yahoo.es).

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The spectrum of cardiovascular anomalies in Costello syndrome includes arteriopathy. *A. Lin[1], L. Bird[2], J.G. Gillessen-Kaesbach[3], P. Grossfeld[2], R. Hamilton[4], D. Hicks[5], M. Innes[6], B. Kerr[7], U. Moog[8], M. Rebolledo[5], K. Vaux[2], D. Wieczorek[3], K. Gripp[9].* 1) Genetics and Teratology, MassGeneral Hosp, Boston, MA; 2) Univ California, San Diego, CA; 3) Institut Humangenetik, Universitatsklinikum Essen, Germany; 4) Hosp for Sick Children, Toronto, Canada; 5) Children's Hosp Orange County, CA; 6) Alberta Children's Hosp, Calgary, Canada; 7) CM and Manchester Children's Hospitals, England; 8) Univ Hosp Maastricht, Netherlands; 9) A.I. duPont Hosp for Children, Wilmington, DE.

Cardiovascular anomalies have been noted in 2/3 of Costello syndrome (CS) patients, of whom about 1/3 each have a cardiovascular malformation (CVM) (usually pulmonic stenosis, PS), cardiac hypertrophy (often hypertrophic cardiomyopathy, HCM), and/or rhythm abnormality (atrial tachycardia). One-fourth have more than one problem. We studied 61 pts and highlight 14 with arterial anomalies. Although a specific gene defect has not been identified in CS, a deficiency of elastin binding protein has been demonstrated. As an elastinopathy, arterial defects would be predicted. PATIENTS: We reviewed 28 new pts (17 enrolled in a natural history plus 11 from individual authors) and 33 well-documented literature cases. Of the total 61, 16 (26%) had a CVM (76% PS), 31 (51%) had HCM and 32 (52%) had a rhythm disturbance (75% atrial tachycardia). Arterial abnormalities were observed in a total of 14 pts including 4 pts with mild aortic root dilation; a boy who died from a stroke at 27 mos. attributed to cerebral vasculopathy; a 6 mos old girl with HCM who died with coronary artery fibromuscular dysplasia; 2 young girls with severe systemic hypertension (HTN), one of whom has pulmonary HTN; a teenage girl with HTN; 3 pts with coarctation; and 2 pts with supravalvar PS. CONCLUSIONS: In this series, HCM and atrial tachycardia were more common and CVMs slightly less common; reporting bias is suspected. We suggest that cardiovascular disease in CS includes arteriopathy with occlusion and dilation of both small and large vessels, a pattern resembling what is seen in Williams syndrome, the prototypic elastinopathy. Additional pts and further analysis are needed to delineate this initial observation.

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Ehlers-Danlos Syndrome Type VIB with Characteristic Facies, Decreased Curvatures of the Spinal Column, and Joint Contractures in Two Unrelated Girls. *T. Kosho[1], J. Takahashi[2], H. Ohashi[3], G. Nishimura[4], H. Kato[2], Y. Fukushima[1].* 1) Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan; 2) Department of Orthopedics, Shinshu University School of Medicine, Matsumoto, Nagano, Japan; 3) Division of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Saitama, Japan; 4) Department of Radiology, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Tokyo, Japan.

Ehlers-Danlos syndrome, kyphoscoliosis type (EDS VI) is an autosomal recessive disorder, characterized by fragile, hyperextensible, and bruisable skin; generalized joint laxity; hypotonia; kyphoscoliosis, and fragility of the globe. The disorder with EDS VI phenotype but normal activity of lysyl hydroxylase is termed EDS VIB, while EDS VIA is the form with deficiency with deficiency of the enzyme. We describe two unrelated girls, aged 11 and 14; with clinical manifestations of EDS type VIB, characteristic facies, skeletal abnormalities, and other features. They had Marfanoid habitus with pectus excavatum; fragile, hyperextensible, and readily bruisable skin with widened, atrophic scars; recurrent hematomas; generalized joint laxity; hypotonia; scoliosis; and mild delay of gross motor development. Lysyl hydroxylase deficiency was ruled out in Patient 1. Parental consanguinity was present in Patient 2. They both had in early childhood down-slanting palpebral fissures, drooping lower eyelids, short nose, small mouth, and long philtrum. Facial features that persisted included thick eyebrows, hypertelorism, strabismus, blue sclerae, low-set and slanted ears, hypoplastic columella, high arched palate, and thin upper lip. They had tubular stenosis of the phalanges, metacarpals, and metatarsals; decreased physiological curvatures of the spinal column with tall vertebrae; and joint contractures including talipes equinovarus and progressive talipes valgus. Their hearing of high-pitched sounds was impaired. They had constipation and recurrent cystitis with an enlarged bladder. In view of these findings, we propose that these two girls represent a clinically recognizable subgroup of EDS type VIB.

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Identification of two novel frame-shift mutations of TOCF1 in Korean patients affected with Treacher-Collins syndrome. *G. Kim[1,2], J. Kim[2], H. Yoo[1,2].* 1) Med Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Genome Research Ctr, Asan Medical Ctr, Seoul. Korea.

Treacher Collins syndrome (TCS; OMIM 154500) is an autosomal dominant disorder of craniofacial development. Clinical characteristics of TCS include malar hypoplasia, micrognathia, downward slanting palpebral fissures, lower eyelid coloboma, malformed auricles, conductive deafness, and cleft palate. Recently, the TOCF1 gene (TOCF1) has been identified as the gene mutated in this craniofacial disease. TCOF1 encodes the nucleolar phosphoprotein treacle that interacts with the upstream binding factor (UBF) and regulates transcription of the ribosomal DNA gene. Using PCR-direct sequencing of the exonic regions of TOCF1, we found two novel mutations, D1379GfsX14 and D1379RfsX14, from two unrelated Korean patients with TCS, both causing a frame-shift mutation at Asp 1379. The D1379GfsX14 and D1379RfsX14 mutations were caused by c.4134_4135insG and c.4134_4135insA, respectively. Both mutations are presumably expected to disturb the regulatory function of TOCF1, deleting the carboxyl-terminal region. Our findings further confirm that functional haploinsufficiency of TOCF1 is responsible for the phenotypic features of TCS. The newly identified mutations will be useful for further understanding of the essential roles of TOCF1 in craniofacial development.

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Rhizomelic shortness of upper limbs, abnormal facial appearance and Congenital Heart defect. *F. Suarez[1,2], L. Martinez[2].* 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Hospital Universitario Clinica San Rafael, Bogota, Colombia.

6 months female, normal term delivery. No medication or alcohol during pregnancy. Healthy unrelated parents. Mother of 16 years old. Birth weight 1,8 kg, Body length: 45 cm. OFC: 34 cm. The clinical examination at birth showed sloping forehead, upturned nose, down-turned corners of the mouth and simple ears. Rhizomelic shortness only of upper limbs, normal hands, fingers and nails. Pectum carinatum umbilical hernia and hipoplasia of the clitoris. The Echocardiogram showed Patent foramen ovale, ventricular septal defect, overriding aorta, and right and left ventricular dilatation. vertebral column and lower limbs were normal. The patient developed heart failure and died. Some of these manifestations overlap with those of Robinow syndrome, but we believe that is a new syndrome with particular abnormalities in face, heart defect and exclusive rhizomelic shortening of upper.

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Microcephalic Osteodysplastic Primordial Dwarfism type I/III in a Mennonite patient: prenatal ultrasound features and further support for an autosomal recessive mode of inheritance. *J.L. Lauzon, F.P. Bernier.* Dept Medical Genetics, University of Calgary, Calgary, AB, Canada.

We present a young girl with Microcephalic Osteodysplastic Primordial Dwarfism (MOPD) type I/III who was born to a consanguineous Mennonite couple. The pregnancy was complicated by severe IUGR and microcephaly detected on prenatal ultrasound at 19 weeks gestation. Other physical features described on prenatal ultrasound included micrognathia, a sloping forehead with a bulbous broad nose and nuchal thickening. Fetal MRI showed a large interhemispheric cyst and agenesis of the corpus callosum. Post-natal physical examination confirmed the prenatal ultrasounds findings and in addition, noted the presence of small palpebral fissures, absence of scalp hair as well as lack of eyebrows and eyelashes, a prominent nasal root and broad nose were noted. She also had contractures of the elbows and shoulders, brachydactyly with tapered fingers, and thick hands and feet. MRI of the brain post-natally showed an interventricular cyst and lissencephaly. The long bones were short and slightly bowed with mild splaying of the metaphyses. Bilateral hip dislocation and horizontal acetabular roofs were also seen on the skeletal survey. Although the patient has been gaining some weight, the prognosis remains guarded due to her severe microcephaly and extensive CNS involvement. This case highlights the possibility for prenatal diagnosis of MOPD I/III and in hindsight MOPD could have been suspected based on the information available prenatally. MOPD is felt to be an autosomal recessive condition based on previous reports of affected children of consanguineous parents. Thus far, no responsible genes have been identified however the occurrence of this condition in a consanguineous Mennonite not only provides further evidence of an autosomal recessive etiology, the identification of further cases in this population may provide an opportunity to identify the responsible gene by using an autozygosity mapping approach.

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Characterization of two cases of partial trisomy 21 due to t(3;21) and t(14;21) translocations. *Y. Kondo[1,2], S. Mizuno[3], K. Yamada[2], Y. Yamada[2], C. Hayakawa[4], T. Ishii[5], N. Wakamatsu[2].* 1) Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; 2) Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi, Japan; 3) Department of Pediatrics, Central Hospital, Aichi Human Service Center, Kasugai, Aichi, Japan; 4) Residental Facilities for Children with Mental and Physical Disabilities, Aichi Human Service Center, Kasugai, Aichi, Japan; 5) Department of Pediatric Psychiatry, Central Hospital, Aichi Human Service Center, Kasugai, Aichi, Japan.

Down syndrome (DS), the most common birth defect associated with mental retardation, delayed development, characteristic facies and congenital heart disease is caused by mostly trisomy of chromosome 21. Although the critical region underlying this syndrome has been refined to a 1.6-Mb region (DS critical region: DSCR) on chromosome 21q22, the underlying molecular mechanism remains unknown. Here, we present two cases of partial trisomy 21 with clinical features distinct from DS. These patients presented with moderate mental retardation and short stature, but the typical facial appearance of DS was not observed. Each patient had a similarly sized extra chromosome 21 by cytogenetic analysis. We performed FISH analysis to examine whether deletions of DSCR might be associated with unusual clinical features in these cases. The results showed that each extra chromosome 21 contained a distal part of chromosome 3p or 14q at the telomeric region of chromosome 21q. The translocation breakpoint of 21q for each patient was located on the centromeric side of DSCR and the sizes of partial trisomy 21 are ~34.5Mb (21pter-q22.1) and ~33.0Mb (21pter-q22.1) respectively. Taken together, these two patients with partial trisomy 21 lack all of DSCR on chromosome 21q22, and their distinct clinical features are likely caused by the genes located at 21pter- q22.1 and the distal part of chromosome 3p (~9.0 Mb) or 14q (~13.3 Mb).

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Mutation study of fibroblast growth factor receptor 2 (FGFR2) gene in Korean kindred with craniosynostosis syndrome. *H.J Kim, S.Y Jeong, J.A Yang, S.J Park.* Dept Medical Genetics, Ajou Univ Col Medicine, Suwon, Korea.

Craniosynostosis is a group of diseases that is generally defined as a premature fusion of one or more cranial sutures. More than 150 syndromes associated with craniosynostosis have been delineated and causative mutations of single genes have been identified in ¡20% of cases. The major genes involved in craniosynostosis encode the transcription factor TWIST and three fibroblast growth factor receptors (FGFRs), FGFR1, FGFR2, and FGFR3. FGFRs are signal-transduction molecules that serve as high affinity receptors for at least 22 fibroblast growth factors (FGFs). FGFRs share a similar sequence structure, characterized by three extracellular immunoglobulin-like domains (IgI, IgII, and IgIII), a single-pass transmembrane segment, and a split tyrosine kinase (TK1/TK2) domain. Mutations in FGFR2, located at 10q26, cause a variety of craniosynostosis syndromes including Crouzon, Pfeiffer, Apert, and Jackson-Weiss syndromes, etc. Molecular basis for the phenotypic variations of FGFR2 gene mutation is not clear. The great majority (94%) of pathogenic FGFR2 mutations are missense in exon IIIa/IIIc, and all confer gain of function to the mutated protein. Few mutations in other regions of FGFR2 including a kinase (TK1/TK2) domain have been reported. In this study, we carried out mutation study of the FGFR2 gene in two Korean kindred with craniosynostosis syndrome. As the results, two point mutations were identified. Missense mutation, C342S, was detected in the exon IIIc of the mutation hotspot region of FGFR2 of 7 years old sporadic case with Crouzon syndrome. The C342S mutation is highly recurrent in Crouzon, Pfeiffer, and Jackson-Weiss syndromes. Another mutation, K641R, was identified in 7 years old familial case with Pfeiffer syndrome and his mother. The K641R mutation in TK2 domain is very rare and only two cases (familial and sporadic) with Pfeiffer syndrome have been reported. These results indicate the allelic heterogeneity of FGFR2 mutations in Korean kindred. With a review of the previously reported cases, clinical variations of patients with FGFR2 mutations were discussed.

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A simple multiplex protocol suitable for screening for *MECP2* mutations in girls with mental retardation. *C.M.B. Carvalho[1], W. Camargo[2], S.D.J. Pena[1].* 1) Biochemistry, UFMG, Belo Horizonte, Minas Gerais, Brazil; 2) Psiquiatra Infantil do Centro Geral de Pediatria, FHEMIG, Belo Horizonte, Minas Gerais, Brazil.

Mutations in the *METHYL-CpG-BINDING PROTEIN 2* (*MECP2*) gene are associated with a pleomorphic clinical picture. Although the most common presentation is Rett syndrome (RTT), *MECP2* mutations have also been found in patients presenting with autism, Angelman syndrome (AS) or less specific forms of developmental delay. This suggests that perhaps all girls with mental retardation should be screened for *MECP2* mutations. However, implementation of such systematic screening using DNA sequencing, with or without previous mutation-detecting techniques, would be extremely time-consuming and expensive. Thus, there is a need for more simple and inexpensive techniques suitable for clinical laboratories, even at the sacrifice of sensitivity. We thus developed a simple PCR-based multiplex minisequencing protocol capable of detecting the most common *MECP2* mutations. This system theoretically allows the detection of the major point mutations and insertion/deletions, especially in exon 4, in approximately 64% of Rett syndrome patients. In most cases the mutation can be diagnosed without need for sequencing. Using this assay we detected mutations in two out of five patients clinically diagnosed as having RTT (a novel *MECP2* frameshift deletion 863_881del and a missense mutation R133C). We also screened twelve patients who had tested negative for Angelman syndrome and found one with a nonsense mutation - 808 CT or R270X. Two of these Angelman patients had a silent, probably polymorphic, mutation 1233 CT. The simplicity and low cost of this methodology qualifies it a potential screening technique for use in girls with developmental delay.

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Bannayan-Riley-Ruvalcaba Syndrome: An unusual presentation with painful vascular malformations in the extremities and Y16 X PTEN nonsense mutation. *R. Klatt, A.S. Teebi.* Div Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada.

Bannayan-Riley-Ruvalcaba syndrome or Ruvalcaba-Myhre syndrome is a phenotypic spectrum characterized by macrocephaly, hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions, and increased risk of developing neoplasm. Variable developmental delay is common and hemangiomas are found in about 10% of the cases. As with Cowden syndrome, this disorder is often caused by mutations in the PTEN gene. We report on a 12 year old girl who presented at the age of 6 years with a limp and about 2 years later had an MRI which showed probable vascular malformation involving her left foot. Around the same time she developed pain and swelling in her left forearm and in the palm of her right hand which on MRI also demonstrated similar findings. A similar lesion was later seen on the left 4th finger. Dermatology assessment was suggestive of blue rubber bleb nevus syndrome. A wedge biopsy of the lesion in her arm demonstrated a vascular malformation. Her manifestations otherwise included macrocephaly with upswept hairline, antimongoloid eye slant, dental malocclusion, leg length discrepancy, and normal intelligence. Family history showed that the father has macrocephaly. No one else in the family had vascular malformations. Two maternal first cousins were born with neural tube defects. Two distant female relatives had had breast cancer in their fifties and sixties. PTEN gene mutation analysis identified a nonsense mutation, TAT to TAA, at codon 16 in exon 1. Because PTEN mutations are known to predispose to cancer, the information has a great impact on the patient and her family. The case presented here demonstrates the importance of studying PTEN mutations in patients with vascular malformations associated with macrocephaly.

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Clinical and cytogenic characterization of a patient with monosomy 1p36 and trisomy 22q13. *R. Saadeh[1], G. Thomas[2], N. Aranyakasemsuk[2], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD; 2) Genetics Lab, Kennedy Krieger Institute, Baltimore, MD.

Deletion of chromosome 1p36, a well-recognized contiguous gene deletion syndrome, is the most commonly observed terminal deletion in humans with a frequency of 1 in 5000 in the general population. Although most (72%) monosomy 1p36 were found to be due to true terminal deletions, 17% were complex and associated with derivative chromosome 1 (Heilstedt et al., AJHG, 72:1200). Clinical phenotypes for most of these complex alterations of 1p36 have not been well characterized. We report a case of a one-year old male with monosomy of 1p36 and trisomy 22q13.3. This child has a history of IUGR, global developmental delay, hypotonia, and infantile spasms. He has plagiocephaly, microcephaly, large anterior fontanelle, posteriorly rotated and low set asymmetric ears, neurosensorial hearing loss, exotropia, a high arched palate, oropharyngeal dysphagia, Ebstein anomaly, cerebral subependymal cysts, and dysgenesis of the corpus collosum. A high-resolution karyotype study revealed 46,XY and a banding pattern suggestive of terminal deletion of 1p36 and possible rearrangement involving 22q. A fluorescent in situ hybridization (FISH) panel for telomeres confirmed that this child was monosomic for 1p36 (probes: CDC2L1, CEB108/T7) and trisomic for 22q13.3 (probe: D22S1726). Studies on his parents showed that his father carried a balanced translocation of 46XY, t(1;22) (p36.3;q13.3) while his mother had a normal karyotype. To our knowledge this is the first reported case of a patient with monosomy 1p36 and distal 22q trisomy, which emphasizes the need for sub-telomere FISH study in all individuals with monosomy 1p36 to identify de novo or inherited chromosomal rearrangements.

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Nail Patella Syndrome: Expansion of the phenotype and first evidence for an association between *LMX1B* mutation position and nephropathy. *E.M.H.F. Bongers[1], F.T. Huysmans[2], G.F. Borm[3], A.M.F. van Remortele[1], J. Schoots[1], L. Hoefsloot[1], R.J.C. Admiraal[4], A. van Kampen[5], H. van Bokhoven[1], E. Levtchenko[6], N.V.A.M. Knoers[1].* 1) Dept of Human Genetics, Radboud University Medical Centre Nijmegen, Nijmegen, Netherlands; 2) Dept of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Dept of Epidemiology and Biostatistics, Radboud University Nijmegen, Nijmegen, The Netherlands; 4) Dept of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5) Dept of Orthopaedic Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 6) Dept of Paediatric Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Nail Patella Syndrome (NPS) is characterized by developmental defects of dorsal limb structures, nephropathy, and glaucoma and caused by heterozygous mutations in the LIM homeodomain transcription factor *LMX1B*. In order to identify possible genotype-phenotype correlations, we performed *LMX1B* mutation analysis and in-depth investigations of limb, renal, ocular, and audiological findings in 106 subjects from 32 NPS families. We detected normal tension glaucoma and sensorineural hearing impairment as new symptoms associated with NPS. Quantitative urinanalysis revealed proteinuria in 21% of individuals. A significant association was identified between the presence of renal involvement in an NPS patient and a positive family history of nephropathy. Sequencing of *LMX1B* revealed 18 different mutations, including 6 novel variants, in 28 families. Individuals with an *LMX1B* mutation located in the homeodomain showed significantly more frequent proteinuria compared to individuals carrying mutations in the LIM-A and LIM-B domains. No clear genotype-phenotype association was apparent for extrarenal manifestations. This is the first study indicating that family history of nephropathy and mutation location might be important in precipitating the individual risk for developing NPS renal disease. We suggest that the NPS phenotype is broader than previously described and that normal tension glaucoma and sensorineural hearing impairment are part of the syndrome.

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Affective states and anxiety among Fragile X premutation carriers. *J. Hunter, E. Allen, A. Abramowitz, M. Rusin, R. Letz, M. Leslie, L. Scott, G. Novak, S. Sherman.* Emory University, Atlanta, GA.

FMR1 contains a CGG repeat sequence in its 5 UTR which results in Fragile X Syndrome (FXS) when it exceeds 200 repeats. Alleles with long unmethylated repeats in the range of ~59-199, termed premutation alleles, have recently been associated with premature ovarian failure in females and a tremor/ataxia syndrome in older males. Both conditions are unrelated to the FXS phenotype. Previous studies have produced conflicting reports of depression and anxiety among premutation females. To further examine these behaviors, we investigated associated measures of depression and anxiety taken from our battery of neuropsychological tests obtained from 82 males and 280 females age 18-50 with varying repeat alleles. The Positive Affect Negative Affect Scales (PANAS) provides measures for positive affect (PA) and negative affect (NA) and the Social Phobia and Anxiety Inventory (SPAI) provides measures for social phobia (SP) and agoraphobia (AP). For preliminary analysis, the subjects were grouped into non- and premutation carriers (< 59 repeats and > 58 repeats) and the mean test scores for the two classes were compared after adjusting for age, gender, race, income, IQ, and education. Subsequently, linear regression was performed using repeat size as a continuous predictor variable. For NA, there was no difference between groups, the adjusted mean for premutation carriers being 2.99 and for noncarriers 2.92. However, repeat size was a significant predictor of NA in the linear regression model accounting for 1.2% of the variance ($p=0.04$). For PA, the adjusted mean for premutation carriers (35.53) was significantly lower than that for noncarriers (36.58) ($p=0.05$). For SP, the mean for premutation carriers (7.54) was significantly higher than the mean for noncarriers (7.31) ($p=0.05$). Similarly, for AP, the mean for premutation carriers (3.71) was significantly higher than the mean for noncarriers (3.55) ($p=0.05$). Repeat size as a continuous variable was not significant in the linear regression model for any of these three measures. Results are consistent with previous reports of increased emotional morbidity associated with premutation carriers.

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CONGENITAL NYSTAGMUS X LINKED: REPORT OF A COLOMBIAN FAMILY. *R. Garcia[1], JC. Prieto[1,2].*
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Patient of twenty two years old, male, from Bogotá (Colombia), assessed for medical genetics in Hospital La Victoria (Bogotá, Colombia). Remitted to medical genetics for neurology with diagnosis of congenital nystagmus. Patient with congenital horizontal nystagmus, initial assessment and management for ophthalmology since two years old until seven years old, with glasses until fourteen years old when suspended voluntarily. He refers remote sight difficulty, adequate near sight, no progression of symptoms. Antecedents of atopical dermatitis, no parental consanguinity, and thirteen male relatives by maternal line with similar disease. On medical examination we found wide nasal bridge, permanent pendular horizontal nystagmus, alternating endotropia, the rest of medical examination was normal. Ophthalmologic assessment reports not corrected, visual sharpness in right eye was 20/400 and in left eye 20/200, color vision was normal, symmetrical horizontal nystagmus, head torsion with sight fixation, increased amplitude with fixation, decrease with convergence and extreme sight, evaluation of anterior segment demonstrate bilateral mild atrophy of iris; bilateral ocular fundus was normal. We made diagnosis of congenital nystagmus X linked. Finally we present a colombian family with fourteen affected men with congenital nystagmus and a clear model of recessive X linked inheritance.

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Anal malformations in a two-generation family with split feet and TP63 mutation. *K. Jedele[1], D. Morash[2], J. Kasser[2], M. Puder[2], K. Marchand[3], V. Kimonis[2,3], D. Levine[3].* 1) Gundersen Lutheran Medical Center, LaCrosse, WI; 2) Children's Hospital, Boston, MA; 3) Beth Israel Deaconess Medical Center, Boston, MA.

Split and/split foot malformation type 4 (SHFM4) and Ectrodactyly-ectodermal dysplasia (EEC) are two in a spectrum of disorders characterized by varying degrees of ectrodactyly, facial clefting and ectodermal dysplasia caused by heterozygous TP63 mutations.

We describe a family referred after prenatal ultrasound showed bilateral foot clefting in a female fetus. The father had similar clefting of both feet, and low anal atresia repaired at two days of life. On examination, he had the clefting of the feet, unilateral 3/4 toe syndactyly, mildly thin and dysplastic nails, but no skin, hair or palatal abnormalities. Postnatal examination of the daughter confirmed the split feet and revealed anterior displacement of the anus without other malformations. Sequencing of exons 5, 6, 7, 8, 13 and 14 of TP63 (GeneDx, Gaithersburg, MD) demonstrated a heterozygous point mutation, K193E, in both father and daughter. This mutation has only been reported once, in a two-generation family with SHFM without other complications. The similar findings in these two families supports the genotype-phenotype correlation seen in Tp63-related disorders.

A literature review found six cases of anal atresia and one of rectal atresia associated with EEC. This report is the first with anal atresia and SHFM, and the first multigenerational anal involvement in TP63-related disorders. In addition, the daughter is the first person reported with anterior displacement, rather than anal atresia. Our new cases indicate that anal malformations may be more common than previously thought in TP63-related disorders, especially as anterior anal displacement may be more easily overlooked than anal atresia. We suggest that evaluation for anal malformations be done on all patients with TP63-related disorders. Finally, these nine cases indicate a role for TP63 in anorectal development.

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Diagnostic Testing of MeCP2 Gene-The Malaysian Experience. *R. Ariffin[1], Y.S. Choy[2], W.T. Keng[2], L.H. Ngu[2], N. Noor[1].* 1) Genetic Unit,Dept of Pathology, General Hospital,Kuala Lumpur, Malaysia; 2) Paediatric Department,General Hospital,Kuala Lumpur,Malaysia.

Rett Syndrome (RTT) is an X-linked neurodevelopment disorder affecting 1/10000-15000 girls. Rett Syndrome causing gene was identified as MECP2 on chromosome Xq28; missense, frame shift and nonsense mutations have been found in the methyl-binding and transcriptional repression domains of the gene in both familial and de novo cases. The phenotype overlaps significantly with that of other disorder such as autism, nonsyndromic mental retardation and idiophatic cerebral palsy. There are rare reports of males born into Rett families who suffered from severe neonatal encelophalophaty and died in early infancy.

The objective of this research is to determine the common mutation pattern in the Retts syndrome cases among Malaysian children. We hope also to identify any novel mutation/ polymorphism among our population.

We extracted DNA from 30 patients suspected to have Rett Syndrome based on clinical features. Then we ran PCR according to optimized protocol. PCR product was run on 1% agarose gel. We proceeded to analyze the ultimate MECP2 gene by focusing on coding region exon 1, 2 and 3. We ran a series of temperature titration on PCR product amplified using primers flanking MECP2 exonic region. on WAVE DHPLC (Denaturing High Performance Liquid Chromatography) against a known wild type sample in 10 cases due to limited resources. Four were identified to carry possible mutation trace of which one is confirmed showing a 16bp deletion on the overlapping region of exon 3d and 3e together with one SNP on exon 2b. The remaining 3 is to be confirmed based on sequencing result.

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Biochemical defects suggestive of mitochondrial dysfunction in Rett Syndrome. *G. Bibat[1,2], L. Kratz[1,2], R. Kelley[1,2], L. Farage[3], A. Horska[3], P. Barker[3], J. Pevsner[1,2], S. Naidu[1,2].* 1) Kennedy Krieger Institute Baltimore, MD; 2) Johns Hopkins School of Medicine Baltimore, MD; 3) Department of Radiology Johns Hopkins Hospital.

Objective: To search for evidence of mitochondrial dysfunction in Rett Syndrome (RTT). Methods: We studied 34 female patients with RTT (ages 1-14 years) with known mutations in the MeCP2 gene. Urinary organic acids (UOA) were quantified by gas chromatography/mass spectrometry. Blood lactate (19/34) and MR Spectroscopy (MRS) (24/34) were also obtained. Results: 11/34 patients had increased levels of UOA in patterns characteristic of mitochondrial disease. These abnormalities were not characteristic of a specific type of mitochondrial dysfunction but were general markers for either primary or secondarily (e.g. redox abnormality) disturbed mitochondrial substrate transport. Blood lactate levels were normal in all subjects including 6/11 patients with abnormal UOA. However, sampling was random and not optimized for detecting milder degrees of diet-sensitive lactic acidosis. MRS showed no elevation of brain lactate in any of the 11 patients studied including 8 with UOA abnormalities. Three patients with increased UOA levels at 3 years of age had normal results at ages 7,13, and 14 years respectively, at which time their blood lactate and MRS results were also normal. UOA abnormalities did not correlate with anticonvulsant use. In this cohort, patients with proximal mutations within the methyl-CPG binding domain (MBD) of the MeCP2 gene were more likely to have UOA abnormalities than those with more distal mutations. Conclusion: We demonstrate mitochondrial dysfunction in RTT as shown by abnormal UOA. However, the basis for these abnormalities remains unknown in view of the normal blood and brain lactate levels. The occurrence of similar mutations in patients with and without urine organic acid abnormalities could reflect the effects of skewed x-inactivation. In patients with the RTT phenotype, the presence of UOA abnormalities should not direct diagnostic testing for a primary mitochondrial disorder to the exclusion of a search for mutations in MeCP2 gene.

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Clinical variability in newborn infants with Beckwith-Wiedemann syndrome. *H. Kawame[1], K. Shimizu[2], T. Nakamura[2].* 1) Div Medical Genetics, Nagano Children's Hosp, Nagano, Japan; 2) Div Neonataology, Nagano Children's Hosp, Nagano, Japan.

Beckwith-Wiedemann syndrome (BWS) is a multi-system disorder characterized by macrosomia (gigantism), macroglossia, and abdominal defects (omphalecele) as major classical features. The etiology of BWS is genetically heterogeneous. Although the molecular genetic testing has been available, the diagnosis of BWS is made primarily on clinical findings. Characteristic features of BWS seem to be evident at birth, but in the clinical setting the definite diagnosis is often difficult in the newborn period. To evaluate the phonotypic spectrum and diagnosis of BWS in the newborn period, we reviewed the findings in 7 patients suspected to having BWS who are ascertain through Genetic Clinics at Nagano Children'fs Hospital from April 2004. The diagnosis was considered based on the criteria described by Shuman & Weksberg.

Average week of gestation at delivery was 36 weeks, and 5 patients were delivered at earlier than 37 weeks. At birth, only 2 patients had birth weight and length above 90th centile, and one had birth weight below 10th centile. Only one patient presented classical triad, i.e. macrosomia, omphalocele, and macroglossia at birth. Four developed macroglossia with age when those had no protruding tongue at birth. One patient with congenital heart defect and not-typical face at birth developed remarkable growth acceleration and macroglossia, and was finally diagnosed as BWS with 11p duplication by chromosomal analysis. Other features included congenital heart defect (2), polydactyly (1), cleft palate (1), inguinal hernia (1), hypothyroidism, and hepatoblastoma (1: at 2-month-old) (1). In summary, classical features are not always appreciated in the newborn infants with BWS. The diagnosis of BWS should be considered for newborn infants with any of associated features of BWS, and the criteria proposed by Shuman & Weksberg is useful for identification of the possible diagnosis of BWS.

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Central nervous system malformations in Townes-Brocks syndrome: a case report. *B. McInnes[1], F. Bernier[1], J. Kohlhase[2].* 1) Dept of Medical Genetics, Alberta Children's Hosp, Calgary, AB, Canada; 2) Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany.

Townes-Brocks syndrome is a pleiotropic multiple congenital anomaly syndrome which has cardinal features of limb, anal and ear malformations. Our patient presented at birth with anterior ear tags, imperforate anus, Grade 2 hypospadias, broad thumbs and 3-4 cutaneous syndactyly of the left foot as well as a hypoplastic 2nd toe. ABR studies done at 4 months of age were completely normal. He was the product of a pregnancy complicated by hyperemesis and pregnancy induced hypertension. A diagnosis of Townes-Brocks syndrome was considered during the neonatal period. Further family history revealed the patient's father underwent minor surgery at birth for a rectal membrane. Audiological evaluation on the father showed a mild to moderate sensorineural hearing loss for the mid to high frequencies in the left ear with normal hearing in the right. The father's parents and siblings are healthy with no congenital anomalies or chronic health conditions. The patient subsequently developed partial complex seizures and migraine headaches for which he is not being treated. MRI revealed a mild Arnold-Chiari malformation type I with no focal abnormalities and increased size of the lateral ventricles. Serial MRI studies showed minimal tonsillar herniation (6mm) at the level of the foramen magnum which has remained stable. CSF cine study showed some restricted flow at the level of the foramen magnum posteriorly and this has also remained unchanged. *SALL1* mutation analysis revealed a frameshift mutation (1815-1816insAA) in both the patient and his father that is previously undescribed but is predicted to introduce a premature stop codon. Other frameshift mutations have been found in the *SALL1* gene and thus, this new mutation is presumed to be pathogenic. Currently, our patient is being monitored for neurological complications of Chiari I. Our case, along with one other TBS patient with CNS malformations, supports the conclusion that these anomalies are a rare finding and further demonstrate the variability in the phenotype and anatomical systems affected by mutations in *SALL1*.

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Healthcare utilization of Down Syndrome infants in Alberta, Canada. *F.P. Bernier[1], M. Ghaffari[2], W. Chang[4], W. Agamah[2], F. Wang[3], L. Svenson[3], T. Chowdhury[2].* 1) Dept Medical Genetics, University of Calgary, AB, Canada; 2) Alberta Health and Wellness Funding and Costing, Edmonton, AB, Canada; 3) Alberta Health and Wellness Health Surveillance Branch, Edmonton, AB, Canada; 4) Dept of Medicine, University of Alberta, Edmonton, AB, Canada.

A pilot study was undertaken in a Canadian Province with a universal health care system to examine the feasibility of using existing population health datasets to derive data on healthcare utilization, co-morbidities and health care expenditure for a cohort of patients with Down syndrome (DS). A cohort of DS infants was obtained from Alberta Congenital Anomalies Surveillance System (ACASS) for 2000-2002. This cohort was linked with four Province of Alberta administrative data sets: hospital inpatient care, ambulatory care, physician claims, as well as the health care insurance registry. All of our 82 DS patients were successfully linked to the inpatient and physician claims dataset whereas linkage to the ambulatory care dataset could not be obtained in 5 patients, likely because of no contact with this part of the health care system. The DS newborns median length of stay (LOS) was 8.5 days, with a quarter staying < 5 days and another quarter staying > 22 days. The LOS was shorter for those from metropolitan regions (5.5 vs. 16 days, p=0.052). Rehospitalization was common is this cohort: 14.1% were re-hospitalized in 30 days, 24.7 % in 90 days, and 34% in 180 days. The most important risk factors for re-hospitalization were cardiac disorders and the gender of the newborns: odds-ratios (90% CIs) were 14.9 (3.7-60.0) for those with vs. without a cardiac disorder; and 0.27 (0.1-0.8) for female vs. male newborns. Further analyses of co-morbidities as well as health care expenditure estimates are also available. Our pilot project demonstrates the feasibility of using comprehensive datasets for assessing the health care utilization of patients with genetic syndromes in order to better understand the natural history, co-morbidities as well as the resources required in order to plan appropriate resource allocation.

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Subtelomere FISH in 79 patients with mental retardation and dysmorphic features detects 9 rearrangements. *A. Battaglia[1,2], A. Novelli[3], C. Ceccarini[3], JC. Carey[2].* 1) Inst Child Neurology & Psych, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Division of Medical Genetics, Dept. of Pediatrics, University of Utah, Salt Lake City, UT, USA; 3) IRCCS-CSS Mendel Institute, Rome, Italy.

Unexplained developmental delay/mental retardation (DD/MR) represents a continuing challenge in clinical diagnosis because in 40% of moderate/severe MR and 70% of mild MR the etiology remains unknown. 5.8% of patients with normal 400-500 band karyotype have subtle subtelomeric rearrangements [Biesecker, 2002]. The yield of new cases identified may increase by preselection based on the degree of MR, physical features and family history [Knight et al,1999; de Vries et al,2001; Riegel et al,2001; Rio et al, 2002]. We performed subtelomere FISH analysis in a cohort of 79 individuals (2 families) with MR, dysmorphic features and a normal karyotype, observed over a 24 month period. Nine subtle unbalanced rearrangements were identified in 13 patients. The findings included three cryptic unbalanced familial translocations; two unbalanced de novo translocations; three de novo deletions; and one de novo duplication. The most common abnormality involved chromosome 1p (3/9). Complex rearrangements were present in 9/13 patients, partial monosomy in 3/13, and partial duplication in 1/13. The time from first normal karyotype to positive subtelomeric FISH result ranged from 3 to 18 years. Six out of 13 cases occurred de novo. The subtelomeric FISH results were useful for adjusting the recurrence risk, giving a better informed patient prognosis, and helping to focus medical screening and monitoring. The results satisfied families in search of a diagnosis, and impacted family planning. The patients with subtelomeric rearrangements showed family history of MR (6/13), prenatal onset growth retardation (11/13), postnatal poor growth (9/13) or overgrowth (2/13), two or more facial dysmorphic features (13/13), one or more nonfacial dysmorphic features and/or congenital abnormalities (13/13). Our findings show a high diagnostic yield (11.4%) of subtelomeric FISH analysis when performed in well selected patients.

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Prader-Willi-Like phenotype: investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. *C.S. D'ANGELO[1], J.A. DA PAZ[2], C.A. KIM[3], D.R. BERTOLA[3], C.I.E. DE CASTRO[1], M.C. VARELA[1], C.P. KOIFFMANN[1].*
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Monosomy 1p36 is one of the most commonly observed mental retardation syndromes in humans that results in a clinically recognizable phenotype including delayed psychomotor development and/or mental retardation (MR), hypotonia, epilepsy, hearing loss, growth delay and/or obesity, microcephaly, brachycephaly, deep-set eyes, flat nasal bridge and pointed chin. Besides, a Prader-Willi syndrome-like phenotype has been described in patients with 1p36 monosomy. Herein, forty-one patients presenting neonatal hypotonia, developmental delay, obesity and/or hyperphagia, behavioral problems and learning disabilities who tested negative for PWS were investigated by FISH and/or microsatellite markers to disclose 1p36 deletions. Twenty-six of the forty-one patients were analyzed with a 1p-specific subtelomeric probe, and one terminal deletion was identified {46,XX.ish del(1)(p36)([TelVysion1p]-)}. Thirty of the 40 patients were investigated by microsatellite markers, and no interstitial 1p36 deletion was found. Our patient presenting the 1p36 deletion did not have the striking features of this monosomy, but her clinical and behavioral features were quite similar to those observed in patients with Prader-Willi syndrome (PWS), except for the presence of normal sucking at birth. Our patients phenotype could be due to the extent of the deletion, (2.5 Mb), smaller than usually seen in monosomy 1p36 patients. Therefore, chromosome 1p36.33 deletion should be investigated in patients with hypotonia, developmental delay, obesity and/or hyperphagia, behavioral problems and learning difficulties who test negative for PWS. Supported by FAPESP, CEPID/FAPESP, CAPES, CNPq.

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Pachygyria and chromosome 22q11 deletion: Cause or coincidence? Report of an additional case. *G. Viot[1], A. Lebbar[1], F. Lewin[2], C. Adamsbaum[3], MC. Nassogne[4], A. Raas-Rothschild[5].* 1) Dept Genetics, Hosp Cochin, Paris, France; 2) Maternity, Hosp Saint Vincent de Paul, Paris, France; 3) Dept Radiology, Hosp Saint Vincent de Paul, Paris, France; 4) Dept Neurogenetics, Hosp Saint-Luc, Bruxelles, Belgique; 5) Dept Human Genetics, Hadassah Hebrew University Medical Center, Jerusalem, Israël.

Pachygyria with 22q11 deletion has been reported (Am J Med Genet : 2003;117(1):80-2; Am J Med Genet : 2004;131(3):322-4) and the question of weather it is a cause or a coincidence raised. Recently, we have the opportunity to diagnose a boy with pachygyria and chromosome 22q11 deletion. At the age of 7 years, this boy showed severe motor and developmental delay (walk and expressive language skills were absent), generalized tonic-clonic seizures, microcephalia (-2,8 SD), behavioral disorders. Clinical examination revealed cupped ears, puffy eyelids, long slender fingers. Neither cardiopathy nor hypocalcemia were present. Brain MRI demonstrated an opercular and frontal pachygyria. No mutation of DoubleCortine gene, no deletion of 17p13.3 region were found whereas a 22q11.2 deletion was detected by FISH analysis using the TUPLE-1 probe. Several central nervous system anomalies have been reported with 22q11 deletion. This observation is an additional case in favour of a relationship between this deletion and pachygyria. cardiopathy or hypocalcemia.

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Rothmund-Thomson Syndrome - Analysis of Genomic Instability in two patients. *S. Tinschert[1], B. Algermissen[2], H. Tönnies[3], H.-P. Berlien[2], H. Neitzel[3].* 1) Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, TU Dresden, Germany; 2) Elisabeth Klinik Berlin, Abteilung Lasermedizin, Berlin, Germany; 3) Institut für Humangenetik, Charité Universitätsmedizin Berlin, Germany.

Rothmund-Thomson syndrome (RTS) (OMIM 268400) is a rare autosomal recessive disorder characterized by photosensitive poikiloderma, small stature, skeletal abnormalities, and juvenile cataracts. It is associated with genomic instability and increased risk for mesenchymal cancers. Mutations in the RECQL4 gene (8q24.3), encoding a protein of the family of helicases, were identified in a subset of RTS patients. Cytogenetic studies identified a somatic mosaicism of either trisomy 8 or isochromosome 8q in a few patients. We analyzed two patients with RTS and their phenotypically normal parents using several cytogenetic techniques including sister chromatid exchange analysis. The two patients showed evidence of increased spontaneous and induced chromosomal breakage. Furthermore, lymphocyte and fibroblast karyoptyping revealed clonal numerical and structural chromosomal abnormalities in particular of chromosomes 8, 7, 9, and 22. A RECQL4 gene mutation was identified in only one of the patients indicating genetic heterogeneity. Teleangiectasias were treated with a flashlamp pumped dye laser (Photognost, BAASEL, wavelength 585nm, pulse 200s, 5,0-6,2 J/cm2) and resulted in complete lesional response. Interestingly, cytogenetic studies of fibroblasts from affected skin after laser therapy showed fewer aberrations than before.

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Unilateral cleft lip and palate with hypotonia and developmental delay with duplication of NIPBL region by microarray analysis. *A.S. Teebi, R. Klatt.* Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

The majority of cleft lip and palate cases are non-syndromic and follow a multifactorial pattern of inheritance. The association of clefting with other even subtle dysmorphic features with or without developmental delay suggests a syndromic association. A subtle chromosomal anomaly might be the underlying cause. We report here a male child assessed first at the age of 1 year as a part of multidisciplinary team assessment and then in follow-up at 2.5 years of age. On initial physical exam the patient was found to have unilateral cleft lip and palate, unilateral left mild epicanthic folds, prominent ears with flashy ear lobule, flat occiput, hypotonia, and mild developmental delay. Routine chromosomal analysis, high resolution chromosomal analysis, SKY and FISH for microdeletion 22q 11 were all normal. The follow-up visit showed height, weight, and OFC to be within normal limits. At this time hypotonia became less prominent and developmental delay became more evident. An ultrasound of the abdomen was normal and FMR-1 testing was negative. Microarray CGH analysis with the SignatureChip^{TM}, which contains 831 clones for 230 genetic loci on 41 chromosome arms, detected an abnormality in the DNA of the specimen. The abnormality is characterized by a gain of a single copy of the three BACs containing the NIPBL region on 5p13. FISH analysis using BAC clones from this region confirmed the duplication. Loss of function mutations in the NIPBL gene result in Cornelia de Lange syndrome (OMIM X608867, 122470). To our knowledge, this is the first description of an individual with a duplication of the NIPBL locus. Microarray analysis of the DNA from both parents was normal which suggests a de novo event in the child. This case demonstrates the importance of searching for subtle chromosomal abnormalities in investigating cases of cleft lip and palate with developmental delay as well as in cases with subtle dysmorphic features and developmental delay. This case also illustrates the usefulness of array CGH in these investigations.

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Molecular characterization and identification of a *de novo* marker chromosome in a child with mild developmental delay. *H.O. Shah[1,3], A. Igleasias[2], B. Miller[1], J.H. Lin[1,3], A. Karnik[1], J. Sherman[1,3], M.J. Macera[4].* 1) Nassau University Medical Center, East Meadow, NY; 2) Div. of Genet., Dept. of Pediat., Beth Israel Medical Center, New York, NY; 3) State University of Stony Brook. Stony Brook, NY; 4) Div. of Mol. Med. & Genet., Dept. of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

The proband at 31/2 years, was referred to genetic services due to a history of mild developmental delays. The family history was unremarkable and his mother denied any use of known teratogenic agents during pregnancy. He was a 6 lb. 10 oz. product of an uncomplicated full term pregnancy and was delivered vaginally without reported complications. Upon examination, his weight was 35.4 lb (25th centile), height was102 cm (75th centile) and his head was 48 cm (<5th centile). He had microcephaly with narrow forehead; mild epicanthal folds; hyoplastic alae nari; bulbous tip and soft ears and mildly decreased muscular tone. Cytogenetic analysis of peripheral blood revealed a 47,XY,+mar karyotype. The marker chromosome was small and unidentifiable with GTG banding. Both parents had normal karyotypes. Comparative genomic analysis (CGH) of DNA from the probands peripheral blood identified additional material on chromosome 8 bands q11.21 to q11.22. Centromeres are suppressed in CGH so FISH analysis with 8(D8Z2) (Vysis) probe was used to verify that the centromere had a chromosome 8 origin. A small detectable signal separate from the D8Z2 signal, was observed with whole painting probe for chromosome 8 (wcp8, Vysis), confirming the presence of 8q11.2. A ring configuration would be the most stable configuration with these breakpoints, so the karyotype was revised to 47,XY,+mar.ish r(8)(p11.1q11.2)(DZ82+,wcp8+). To our knowledge, this is the first reported case in the literature of an additional r(8) with these breakpoints. The use of CGH coupled with FISH now provides a method for the identification of such minute marker chromosomes. Identification and classification will lead to a better understanding of the clinical manifestations caused by these markers, thus providing improved treatment for those affected individuals.

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Ring chromosome syndrome: report of 12 cases. *B. Vasconcelos[1], L.M.J. Albano[1], C.I.E. Castro[2], D.R. Bertola[1], I.C. Sbruzzi[1], C. Koiffmann[2], C.A Kim[1].* 1) Genetics Unit, Instituto da Criança, São Paulo, Brazil; 2) Bioscience Institute - São Paulo University, São Paulo, Brazil.

Ring chromosomes result from terminal breaks and a subsequent fusion of both arms of the same chromosome or a telomere dysfunction. The clinical features depend on the size of the material lost. Ring syndrome (Cotè et al., 1981) is characterized by growth delay, no major malformations, minor anomalies and mental retardation, independently of the chromosome involved. We decided to study 12 ring chromosome cases in order to delineate their phenotype. Five of the cases were mosaic (42%), 3 of them confirmed by FISH technique. The patients age ranged from 2mo-18y, involving the following chromosomes: 2 (1); 4 (1); 5 (1); 10 (1); 13 (1); 18 (2); 22 (2); X (1); and two rings were not identified. The clinical features were: short stature - 11/12 (92%); developmental delay/mental retardation - 10/11 (91%); craniofacial dysmorphysms - 10/12 (83%), specially dysplastic ears - 8/12 (67%); speech delay - 9/11 (82%); microcephaly - 6/12 (50%); skeletal abnormalities - 4/12 (33%); strabismus and genitourinary anomalies 3/12 (25%); and central nervous system anomalies - 2/12 (17%). Our findings were in accordance with that of the literature. Cutis girata, present in one 22 ring chromosome case, confirmed by FISH, has already been previously reported, suggesting that this finding could be specially associated with 22 ring chromosome. The high frequency of dysplastic ears in our sample (67%) may indicate that this sign is the most important minor anomaly in ring syndrome. Major malformations such as central nervous system and genitourinary anomalies were also detected in our cohort.

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PRESENTATION OF TWO COUPLES WITH BALANCED RECIPROCAL TRANSLOCATION AND RECURRENT ABORTION THAT INVOLVE TO THE CHROMOSOME 4. *G. Noceda-Rivera, G. Razo-Aguilera, R. Baez-Reyes.* Deparment of Genetics, National Institute of Perinatology. Mexico,City.

The incidence of a chromosomal arrangement is of 1 in 500 individuals of the general population,is so most of the carrier of balanced reciprocal translocation, they are normal in their phenotype, detected during citogenetic studies due to problems in the reproduction, such as recurrent abortion, previous descendence with birth defects or sterility. We present two cases that we saw in the Clinic of Genetics of the Reproduction and Prenatal Diagnosis. CASE 1: Female patient of 29 years old, 31 years old her couple, not consanguineous, G-IV P-I A-II, the karyotypes with result of the patient: 46,XX t(4;6)(p10;p10) and her husband: 46,XY, the family study was completed. CASE 2: Female patient of 36 years old, 33 years old her couple, not consanguineous, G-IV C-II P-I A-III, she went to the consultation of prenatal diagnosis with 16 weeks gestational age by maternal age and recurrent gestacional loss, the karyotypes resulted for the patient: 46,XX and her husband: 46,XY t(4;9)(p14;q22), we offered amniocentesis being reported karyotype 46,XX, the family was completed.The correlation of the break points with the genes involved in both cases was made. Is necessary an appropiate genetic advice for translocations carriers, that imply to determine the risk based on the carrier member, involved chromosomes, the segregation form and the family genetic history, that which allows to calculate the reproductive risk with more certainly.

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Short stature in patients with 47 XXY- an unusual coexistence of another genetic syndrome. *L. Ngu[1], Y. Choy[1], W. Keng[1], A. Ruziana[2], M. Aminah[2].* 1) Genetic Unit, Pediatric Inst, Kuala Lumpur Hosp, Kuala Lumpur, Malaysia; 2) Cytogenetic laboratory, Kuala Lumpur Hospital.

Klinefelter syndrome, or 47 XXY is the commonest sex chromosome disorder affecting 1 in 750 to 1000 males. They are usually not suspected in children as the classical features of the syndrome usually manifest in mid or late adolescence. They may be diagnosed if karyotyping is done for children with learning difficulties, language disorders or attention deficits. Most of the individuals with 47 XXY are normal in height in the first 5 years of life and height velocity increased after 5. They have tall stature by adolescence. Therefore, it would be most surprising to know the result of karyotyping done for the evaluation of a child with short stature.

We report here 2 patients identified to have 47 XXY and another syndrome. One of them was diagnosed having Noonan syndrome as he had short stature, webbing of the neck, sparse hair, hypertelorism, epicanthal folds, down-slanting palpebral fissures, flat nasal bridge, low set ears and small chin. He also had a small patent ductus arteriosus and ventricular septal defects which closed spontaneously. His bone age was delayed and his height was falling off percentiles chart. There was a deficient response in growth hormone stimulation test.

The other patient was referred for a suspicion of skeletal dysplasia because of short stature and mesomelic shortening. He had Robinow syndrome with hypertelorism and prominent eyes, flat and short nasal bridge, upturned nares, broad thumbs and big toes and bilateral cryotchidism. Skeletal survey revealed hemivertebra at T8. Growth hormone stimulation test showed a normal response. Both the patients had 47 XXY in 100% of their blood and skin cells tested. Deletion in the SHOX gene was ruled out by FISH. Since 47 XXY is a relatively common condition, the coexistence of another genetic syndrome is the most plausible explanation for short stature in these individuals. There were 2 previous reports on 47 XXY associated with Noonan syndrome and Russell syndrome.

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Severe hypotonia and SMA in an infant with an unbalanced chromosomal complement. *S. Ben Shachar, L. Potocki, S.R Lalani.* Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, Houston.

Unbalanced chromosomal translocations occur with a frequency of 1:2500 and are often characterized by dysmorphic features and multiple congenital anomalies. Hypotonia can also be a feature in these patients, yet is also seen in several single gene disorders. We present a patient with multiple congentital anomalies, dysmorphic craniofacial features, severe hypotonia, and absent deep tendon reflexes, who presented at age 4 months with respiratory distress requiring mechanical ventilation. Imaging studies including brain MRI and head CT scan revealed lambdoid, coronal and sagittal craniosynostosis without definite brain parenchymal abnormalities. G-banded chromosome analysis revealed an abnormal male complement: 46,XY,der(3)(3;7)(p25;q36). Chromosomal microarray analysis confirmed this cytogenetic abnormality and also revealed trisomy of the Sonic Hedgehog (SHH) locus. In reviewing of the medical literature, we found that the features in our patient were unique as compared to other patients with chromosomal aberrations of these regions. Furthermore, although chromosome analysis and CMA revealed an unbalanced translocation, the severity of the hypotonia prompted further clinical investigation. Muscle biopsy and molecular analysis of this patient confirmed an additional diagnosis of spinal muscular atrophy type I (SMA). Chromosome analysis and DNA analysis of the parents are pending. This case presentation exemplifies the need for continued diagnostic testing in patients with unusually severe presentations. Molecular testing in this family allowed for more accurate counseling regarding prognosis in this patient and recurrence risk for future pregnancies, thus enabling informed decision making.

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Is it Alagille or William syndrome? The importance of microarray studies in difficult cases. *P. Shah[1], P. Murphy[1], D.L. Skidmore[2], S. Scherer[2], W. Halliday[3], D. Chitayat[2,4].* 1) Dept Pediatrics, Mount Sinai Hosp, Toronto, ON, Canada; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Pathology and Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; 4) Prenatal Diag & Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada.

Both Williams syndrome and Alagille syndrome are difficult to diagnose at birth, as the characteristic facial dysmorphisms may not be present.The main findings of Alagille syndrome in the newborn include dysplastic pulmonary valves, vertebral abnormalities, conjugated hyperbilirubinemia. We report a newborn with findings suggestive of Alagille syndrome that was found to have Williams syndrome. He was born by C-section for severe IUGR at 31 weeks. Amniocentesis revealed a normal male karyotype. In keeping with Alagille Syndrome, the baby developed direct hyperbilirubinemia on day 2 of life with elevated liver enzymes. Echocardiogram revealed proximal left pulmonary artery narrowing and a small VSD. X rays revealed butterfly vertebrae. In addition, in the first week of life, there was an unexplained period of diffuse hyperpigmentation in a Caucasian child, which waned after several days. The baby had an acute deterioration on day18 and died. Autopsy revealed renal and pulmonary abnormalities, and microcephaly with partial agenesis of corpus callosum. Bile ducts were identified, making Alagille syndrome less likely. Concordantly, microarray comparative genomic hybridization (CDG) demonstrated the absence of a microdeletion at the Alagille locus, and instead demonstrated a microdeletion at 7q11.23. FISH confirmed the unexpected diagnosis of Williams syndrome. This case further demonstrated the importance of microarray CDG in the neonatal period in assisting in the diagnosis of conditions for whom that characteristic dysmorphisms may not be apparent in the first months of life. In this case, the diagnosis of Williams syndrome enabled the provision of accurate counseling which otherwise would have not been possible. In light of the atypical features for Williams Syndrome, attempts are underway to further delineate the size of the deletion.

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No causative genomic aberrations by BAC microarray CGH in forty patients with Kabuki make-up syndrome.

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Kabuki make-up syndrome (KMS) is a congenital anomaly disorder characterized by specific facial features, mild to moderate mental retardation, postnatal growth delay, skeletal abnormalities, and unusual dermatoglyphic patterns. The multisystem involvement of the KMS phenotype suggests that KMS is caused by a microdeletion or microduplication involving several genes. Microarray using 2,173 BAC clones covering the whole human genome has been constructed. All clone spotted were confirmed to show a unique signal at the predicted chromosomal location by in-house FISH analysis. A total of 40 KMS patients were analyzed by BAC microarray CGH (BM-CGH). In two KMS patients, visible cytogenetic chromosomal abnormalities could be precisely detected by BM-CGH. In addition, microdeletions at 39 clones and duplications at 145 clones in 40 KMS cases were identified by BM-CGH and were confirmed by FISH, but most of them were not considered to be responsible for the pathogenesis of KMS because the same copy changes were observed in normal control individuals. One deletion at 2p11.2 and one duplication at Xp22.3 found in KMS may be related to the phenotype as they were not observed in normal controls, although this remains inconclusive. The constructed microarray was proved to be reliable for the detection of known chromosomal rearrangements in KMS patients, but no uniform rearrangement in KMS could be detected. Thus KMS is unlikely to be a microdeletion/duplication syndrome.

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Involvement of chromosome 7q31 region in the Russell- Silver syndrome. *V. Adir[1], S. Shalev[2], Y. Hujeirat[2], A. Shalata[1], Z.U. Borochowitz[1].* 1) The Simon Winter Institute for Human Genetics, Bnai-Zion Medical Center, Technion-Rappaport Faculty of Medicine, Haifa, Israel; 2) Department of Genetics, HaEmek Medical Center, Afula, Israel.

Russell- Silver syndrome (RSS) is a clinically and genetically heterogeneous developmental disorder involving growth retardation and a characteristic small triangular face. Chromosome 7 has been strongly implicated in RSS cases. Several cases involving reciprocal translocation, matUPD(7), and segmental deletions have been reported. However the specific region responsible for the disorder is unclear and the mechanism which causes this condition remains unknown. Here we report on a young male suspected of possessing RSS. Pre and post natal growth retardation was identified. Clinical examination revealed sparse hair, small-triangular facial features, high-wide forehead, low rotated ears, hypoplasia of the mid-phalanges of fingers as well as clinodactyly of the fifth finger. Molecular testing of polymorphic markers along chromosome 7 revealed an absence of paternal contribution alleles at D7S1817 and IVS17BTA. Our results suggest a de novo interstitial deletion of chromosome 7 at band q31 of about 12Mb. A second, less likely scenario, would entail a segmental matUPD(7) at that region. The proximal breakpoint is located at 7q31.1 between the polymorphic makers D7S692 and D7S1817 and the distal breakpoint which is located at 7q31.3 between marker D7S487 and the CFTR gene. Screening the suspected deleted region for candidate genes reveals few genes which could be clearly indicated as playing an important role in cell growth and development. A previous report on duplication of this region in a patient with mild facial dysmorphism and a moderate mental retardation together with UPD(7) in RSS suggests dosage sensitive mechanism of this region in growth and developmental processes. We suggest that this region should be taken into consideration as being responsible for the clinical features of this patient and possibly for the RSS phenotype.

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A 2.5 Mb deletion in the 19p13.2 region detected by CGH-array in a boy with mental retardation, behavioural troubles and short stature. *A. Jacquette[1], R. Gesny[1], M. Rio[1], O. Raoul[1], M. Vekemans[1], A. Munnich[1], N.P. Carter[2], V. Cormier-Daire[1], L. Colleaux[1].* 1) Department of medical genetics and Inserm U 393, Necker Hospital, Paris, France; 2) Wellcome Trust Sanger Institute, Hinxton, U.K.

CGH-array is a new tool to describe small rearrangements of chromosome that have never been reported before. Here, we report the case of a 12-year-old boy with a 19p13.2 deletion revealed by CGH-array. He was first seen at the age of 2 for behavioural troubles with hyperactivity, self injury and heteroaggressivity . He had a pre and post natal growth retardation (weight and height on -5 SD), a psychomotor delay (walk at 30 months of age, a few words at 12 years of age), some facial dysmorphic features with hypertelorism, prominent forehead, short philtrum, large mouth and low-set ears with thick lobes and hypospadias. In addition, MRI showed a complete absence of corpus callosum. The first screening (including metabolic and chromosomal investigations) was normal. Based on the behavioural troubles, the dysmorphic features and the severe mental retardation, he had benefited from a CGH-array analysis (3 523 clones, provided from the Wellcome Trust Sanger Institute) and this investigation led to the identification of a 2.5Mb deletion in the 19p13.2 region. Microsatellites analysis confirmed the absence of paternal contribution. To our knowledge, this is the first case report of a so small deletion in 19p13.2 region in association with a severe mental retardation with behavioural troubles, short stature and dysmorphic features.

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Molecular Diagnosis in fetuses with Holoprosencephaly : microdeletions have major occurrence. *C. Bendavid[1,2], C. Dubourg[1,2], I. Gicquel[1], P. Saugier-Veber[3], M.R. Durou[2], L. Pasquier[4], S. Jaillard[5], B.R. Haddad[6], C. Henry[5], T. Frebourg[3], S. Odent[4], V. David[1,2].* 1) Faculte de Medecine, Rennes1, UMR 6061 CNRS, Rennes, France; 2) Laboratoire de Genetique Moleculaire, CHU Pontchaillou, Rennes, France; 3) Laboratoire de Genetique, INSERM U614, CHU Rouen, France; 4) Genetique Medicale, CHU Hopital Sud, Rennes, France; 5) Laboratoire de Cytogenetique, CHU Pontchaillou, Rennes, France; 6) Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA.

Holoprosencephaly (HPE), the most common structural malformation of the forebrain in humans, can be early detected in fetuses during pregnancy by prenatal ultrasound. But, among these HPE fetuses with normal karyotypes, only 14% have point mutations in known HPE genes (SHH, ZIC2, SIX3 and TGIF). As shown previously, life-born infants with HPE have 22 % point mutations but also 4% large rearrangements detectable by FISH and real time quantitative PCR (qPCR) in HPE genes. Consequently, we hypothesized that microdeletions should also be involved in HPE fetuses that usually have more severe phenotypes or associated malformations. We screened DNA from 73 HPE fetuses that had normal karyotypes and no mutations in four HPE genes (SHH, ZIC2, SIX3 and TGIF) for the presence of microdeletions. Real time quantitative PCR assays were employed for rapid determination of HPE genes copy numbers and identified microdeletions were confirmed by FISH or Quantitative Multiplex PCR of Short Fluorescent Fragments analysis (QMPSF). Seven microdeletions in a total of 73 samples were detected : 2 in SHH, 2 in SIX3, 2 in ZIC2 and 1 in the TGIF gene. Thus, we identified microdeletions in 9.5% of prenatal HPE samples compared in 4% in postnatal HPE samples. These data suggest that microdeletions are a common cause for prenatal HPE. Their detection can be best achieved by the QMPSF testing method that proved to be the most efficient for testing several genes in a single assay.

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Y-chromosome microdeletions in patients with 45,X/46,XY gonadal dysgenesis. *F. Alvarez Nava, H. Puerta, M. Soto, A. Rojas-Atencio, L. Pineda.* Unidad de Genetica Medica, Univ del Zulia, Maracaibo Zulia, Venezuela.

Introduction: Among the disorders with numerical abnormalities of the Y-chromosome is 45,X/46,XY mosaicism. Its clinical manifestations include varying degree of impairment of testicular development. The commonest structural abnormalities of Y-chromosome are microdeletions of the long arm (Yq) in one or more of the three AZF (Azoospermic Factor) regions, AZFa, b or c. Deletions involving one of these three AZF loci result in the loss of multiples genes normally expressed in testicular embryonic development and adult testicular tissue and so they are associated with spermatogenic failure. Objective: Since deletions of AZFc region has been related to 45,X/46,XY mosaicism we studied whether microdeletions of AZF loci could affect the clinical and gonadal phenotype in patients with 45,X/46,XY karyotype. Materials and methods: We screened for Y-chromosome microdeletions on DNA isolated from both peripheral blood lymphocytes (PBL) and gonads of 11 Venezuelan patients with 45,X/46,XY mosaicism. Y-microdeletions analyses were performed using a multiplex polymerase chain reaction (PCR)-based technique with 20 sequences-tagged-sites (STSs). Results: Three patients had Y-microdeletions on PBL-DNA and five patients had Y-microdeletions on gonadal-DNA. Although more and larger Y-microdeletions were found on DNA-gonadal than DNA-PBL among our patients, there is not a correlation between Y-microdeletions and severity of phenotype. Conclusions: These data are compatible with a sequence-model in which the primary alteration in 45,X/46,XY mosaicism is a molecular defect in Y-chromosome specific DNA-sequences that result in the mitotic loss of Y-chromosome. Therefore, the global Y-chromosome instability is detected at DNA-level as partial and interstitial Y-chromosome microdeletions, and at cytogenetic-level as a 45,X/46,XY mosaicism.

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MLPA is a highly effective diagnostic method in 22q11.2 microdeletion syndrome. *L. Fernández[1], P. Lapunzina[1], D. Arjona[2], D. Elorza[3], L. García-Guereta[4], I. López Pajares[1], M.L. De Torres[1], M.A. Mori[1], M. Palomares[1], M. Burgueros[4], J. Pérez[3], A. Delicado[1].* 1) Department of Medical Genetics, Hospital Universitario La Paz, Madrid, Spain; 2) Sequencing Unit, Hospital Universitario La Paz, Madrid, Spain; 3) Department of Neonatology, Hospital Universitario La Paz, Madrid, Spain; 4) Department of Pediatric Cardiology, Hospital Universitario La Paz, Madrid, Spain.

22q11.2 deletion syndrome is widely diagnosed by commercial fluorescence in situ hybridization (FISH), the chromosomal breakpoints and size of the deletions mostly determined either by polymorphic STR markers segregation tests or by specific hybridizations. Multiplex Ligation-dependent Probe Amplification (MLPA) can be a useful resource to detect haploinsufficiency in 22q11.2 and other chromosomal regions associated with DiGeorge/velocardiofacial syndrome in a single assay. We have compared the results of the three techniques on a group of 30 patients affected with 22q11.2 deletion syndrome and previously diagnosed by FISH. MLPA was successful in diagnosing all the patients, and in regard of the deletion size, the MLPA results were concordant with the STRs for all the patients and they resolved 7 cases that were uncertain by STRs due to uninformative results, lack of parental data or problems in markers amplifications. Other copy number variations can also be detected in patients with apparently no typical deletions. This supports the usefulness of the MLPA as a diagnostic tool for 22q11.2 microdeletion syndrome, as a rapid, easy, feasible and high-throughput method.

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Natural history of trisomy 18 fetus diagnosed in the third trimester. *K. Kurosawa, Y. Igarashi, T. Yamamoto, K. Sameshima, T. Setoyama, M. Takei, T. Hirabuki, M. Yamanaka, Y. Kuroki.* Kanagawa Children's Med Ctr, Yokohama, Japan.

Trisomy 18 is the second most common autosomal trisomy in liveborn infants, and occurs at a frequency of 1 per 3,000 - 5,000 live births. Conbined with the ultrasonographic examination, multiple marker screening test involving the maternal serum AFP, uE3, and hCG in second trimester was reported to identify about 60% of trisomy 18 with a 0.4% false positive rate. The life expectancy of trisomy 18 after live born reveals the high infant motality rate . About 90% children with trisomy 18 die before their first birthday. Recently the natural history of trisomy 18 of live born was investigated and reviewed extensively. However, there is still a lack of precise data about their natural history in the cases diagnosed prenatally in third-trimester. To delineate the natural history of 18 trisomy fetus prenally diagnosed in the third trimester, we reviewed the 50 cases diagnosed after 27 weeks of gestation in our institute between 1994 and 2003. As the control subjects, we compared the life expectancy, complications, and sex ratio with those of transferred from other hospitals after live-born. 57% of the cases were live births, and 43% were stillbirths. Prenatally diagnosed cases reached to live-born showed no difference in the prognosis and life expectancy to the control group. The sex ratio was about 1 at that time of prenatally diagnosed, but rapidly declined at term. These information was valuable for counseling to families with the prenatally diagnosed cases and will provide insight into the sex ratio of the disorder.

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A duplication of distal Xp associated with hypogonadism, hypoplastic external genitalia, mental retardation and congenital abnormalities. *L. TELVI[1], M. MINZ[1], C. BELLESME[2], J.M. DUPONT[3], P. BOUGNERES[2].* 1) Cytogenetics Laboratory, Hospital St Vincent de Paul,; 2) Endocrinology Department, Hospital St Vincent de Paul; 3) Cytogenetics Laboratory, Hospital Cochin, Paris, France.

An unusual case of a boy presented with a partial duplication of distal Xp sequences is described. The proband, an 7 year old boy, showed mental retardation, dysmorphic features, hypogonadism, hypoplastic external genitalia, short stature and hypotrophy. His karyotype was 46,Y,dup(X)(p21.2-22.3). The proband's mother showed the same karyotype. We used a panel of probes located in the short arm of the X chromosome for the identification of chromosomal anomaly. In 1996 we published a case with an inv dup(Xp) and similar phenotype. However, this kind of cases are rares. Their study is usefull for specially prenatal genetic counceling. We compared this two cases and the other cases published in the litterature.

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A possible locus for Fryns syndrome at 1q41q42. *B. Pober[1,2], C. Prada[4], F. Blaise[3], T. Boyd[3], R. Jennings[1], L. Wilkins Haug[5], J.P. Fryns[6], C. Lee[5], S. Kantarci[2], D. Casavant[2], P.K. Donahoe[2], V. Kimonis[4].* 1) Dept Surgery, Childrens Hosp, Boston, MA; 2) MassGeneral Hospital for Children, Boston, MA; 3) Dept Pathology, Childrens Hospital, Boston, MA; 4) Division Genetics and Metabolism, Childrens Hospital, Boston, MA; 5) Dept Pathology or Obstetrics, Brigham Womans Hospital, Boston, MA; 6) Center for Human Genetics, University Hospital of Leuven, Leuven, Belgium.

Fryns syndrome, the most common syndrome associated with congenital diaphragmatic hernia (CDH), is presumed to be autosomal recessive based on reports of affected siblings and parental consanguinity. The phenotype is broad but cardinal features include: distinctive facial dysmorphology, CDH, pulmonary hypoplasia, distal digital/nail hypoplasia, and one or more characteristic major malformations. We report a 1q41q42 microdeletion detected by CGH in a case clinically diagnosed with Fryns syndrome.

A female infant, twin B, was born at 37 weeks. Placenta was dichorionic diamniotic; amniocentesis was 46,XX from both sacs. Twin B experienced respiratory distress, was difficult to intubate, and died at 1 hour. An autopsy demonstrated: coarse dysmorphic facies, enlarged fontanelles, cleft palate, distal phalangeal and nail hypoplasia, large left CDH, mild right diaphragm eventration, pulmonary hypoplasia, possible laryngeal stenosis, and a VSD. Karyotype on fetal skin was normal. Twin A is healthy and nondysmorphic at 3 months of age.

Application of array-based Comparative Genomic Hybridization using the Spectral Chip 2600 demonstrated deletion of 3 clones mapping to 1q32.3-1q42.2. Deletion involving 1q41q42 was independently confirmed by FISH and by use of the SignatureChip which showed deletion of 9 clones. Cytogenetic abnormalities in or near this chromosome band have been previously reported in CDH patients. Our finding suggests that deletion or mutation of a gene in this locus can lead to CDH, and possibly Fryns syndrome. This finding also indicates that Fryns syndrome may be etiologically heterogeneous since all cases appear not to follow an autosomal recessive pattern of inheritance.

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Pursuing the identification of DURS1 gene. *H. Baris[1,3], W-M. Chan[1], C. Andrews[1,3], J. Ranells[4], T. Pal[5,6], E.C. Engle[1,2,3].* 1) Dept of Medicine (Genomics), Children's Hosp, Boston, Boston, MA; 2) Dept of Neurology, Children's Hospital Boston, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Department of Pediatrics University of South Florida; 5) H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; 6) The University of South Florida, College of Medicine, Department of Interdisciplinary Oncology and Pediatrics, Tampa, Florida.

Duane syndrome (DS) is a genetically heterogeneous strabismus disorder with horizontal gaze restriction and globe retraction. There are 3 identified DS loci. DURS1 was cytogenetically mapped to 8q13; DURS1 patients have sporadic DS accompanied by other clinical findings. In 2002, Pizzuti et al. demonstrated disruption of CPA6 in a DS patient carrying a de novo reciprocal balanced translocation t(6;8)(q26;q13); 18 sporadic DS patients did not harbor mutations in the CPA6 gene. To further investigate the role of CPA6 in sporadic DS, we have screened 106 sporadic Duane syndrome patients by denaturing high-performance liquid chromatography. We find no coding mutations in CPA6. Next, we identified a boy with DS, high-frequency hearing loss, velopharyngeal incompetence, decreased upper body strength, fine motor in coordination, learning disabilities, and attention deficit disorder. FISH for velocardiofacial syndrome on 22q11 was normal and chromosomal analysis revealed 47,XY,inv(8)(p11.1q13.1)+mar[5]; 46,XY,inv(8)(p11.1q13.1)[1]. Parental chromosome analyses are not available. M-FISH and FISH with chromosome 8 centromeric probe confirm that the marker chromosome contains chromosome 8 material. We performed further FISH analyses and demonstrate 1) the mosaic marker chromosome contains duplication of the centromeric breakpoint region of 8q, encoding at least two known genes; 2) the telomeric breakpoint is on band 8q13.2 flanked by BAC clones RP11-50A22 and RP11-779P1 and containing VEST1. VEST1 is expressed in the inner ear, and is of unknown function. Although we cannot exclude the potential role of the mosaic marker chromosome in the pathogenesis of our patient's disorder, VEST1 is a DS candidate gene.

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Array-CGH for the identification of autism susceptibility loci. *M.R. Mansouri[1], A-C. Thuresson[2], C. Betancur[3], M. Leboyer[3], C. Gillberg[4], C. Langford[5], N. Dahl[1].* 1) Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2) Clinical Genetics, Uppsala University Children's Hospital, Uppsala, Sweden; 3) INSERM U513, Faculty of Medicine, University of Paris XII, Créteil, France; 4) Dept of Child and Adolescent Psychiatry, Göteborg Universitet, Göteborg, Sweden; 5) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom.

Autism is characterised by impairment in reciprocal social interaction and communication and restricted and stereotyped behaviour with developmental abnormalities which appear in the first three years of life. The prevalence for autism is around 0.5% of the general population. There is strong evidence supporting genetic components behind idiopathic autism. Recent studies have indicated constitutional large-scale copy number alterations in patients with mental retardation using microarray based comparative genome hybridization (array-CGH). We hypothesise that genomewide array-CGH will allow for the identification of gene regions and genes involved in normal cognitive development by studying patients with autism. Using 1 Mb array-CGH, we have initiated screening of three cohorts of patients including 60 sib-pairs with autism, 50 singleton cases with autism only and 100 patients with autism and associated features such as dysmorphism sampled in France and Sweden. The results of these ongoing studies will be presented and discussed.

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Novel findings in 46,XY females with partial gonadal dysgenesis using a targeted CGH based clinical microarray.

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In man, male sex is determined by the dominant action of the primary testis determining gene, Sry, located on the Y chromosome. Deletions or mutations of Sry are known to lead to XY sex-reversal. However, a significant number of cases of XY female sex reversal do not involve the Y and are associated with autosomal chromosomal rearrangements. These include deletion of terminal 9p, distal 10q, 11p, 17q and duplications of 1p and Xp. For the most part these patients have not been systematically studied and the critical deletion intervals were poorly defined. We have previously reported a chromosomal microarray analysis (CMA) for quickly and efficiently identifying chromosomal rearrangements. We have applied this targeted chromosomal microarray for CGH based analysis of clinically relevant chromosomal disorders and subtelomeric regions to two XY female patients with gonadal dysgenesis. Case 1: previously described with a complex rearrangement with two clonal populations i.e. 46,XY,t(9;14)(p24;q11) and 46,XY,t(9:14)(p24;q11),+r(14) (Genomics 42:271,1997). A deletion was detected at the molecular level from 9p24 to 9pter. CMA indicated the patient harbored deletions of 4 clones spanning 2.2 Mb of genomic DNA corresponding to the 9p24.3 region. Case 2: previously described with an apparently normal 46,XY chromosome complement using high resolution karyotype analysis. A deletion of distal 9p24 was detected by molecular analysis of microsatellites (Am J Hum Gent 63:901, 1998). The CMA revealed a loss of 2 clones encompassing a 1 Mb region on the distal short arm of 9p24.3. In addition, CMA indicated a gain of 2 clones encompassing approximately 0.5 Mb region on the distal short arm of 7p22 that was previously undetected. The major advantages of this selected human genome array include: interrogation of clinically relevant genomic regions, the ability to test for a wide range of duplication and deletion syndromes in a single analysis.

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Phenotype of cryptic subtelomeric 4q deletions in 4 patients of different age. *M. Edwards[1,2], K. Fagan[3], G. Peters[4], A. Hackett[1,2].* 1) Hunter Genetics, HNE Health Service, Waratah, NSW, Australia; 2) University of Newcastle, Australia; 3) Cytogenetics, Hunter Area Pathology Service, Newcastle; 4) Cytogenetics, Children's Hospital at Westmead, Australia.

Four female patients with moderate to severe intellectual disability were found to have 4q subtelomeric deletions on screening by FISH, after normal routine blood karyotyping. Three had a similar phenotype.

Clinical Feature	Patient DM	MR	CL	KL
Dolichocephaly	+	+	+	-
Craniosynostosis	-	+	+	-
Sagittal ridge	+	+	+	-
Ptosis	+	+	+	-
Long narrow face	+	+	+	-
Gothic palate	+	+	+	-
Tall/thin build	+	+	+	-
Macrocephaly	+	+	+	+

DM had a de novo 4q deletion. CL and her niece MR had an unbalanced reciprocal t(4;15). Some features of DM, MR and CL are seen in Shprintzen-Goldberg syndrome, although none had arachnodactyly. Patient KL aged 39 had short stature, seizures, coarse face, cataracts, glaucoma, brachydactyly. Detailed FISH deletion mapping will be presented.

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TEN YEARS OF EXPERIENCE OF THE CLINIC OF GENETICS OF THE REPRODUCTION IN THE NATIONAL INSTITUTE OF PERINATOLOGY. *R. Baez-Reyes, G. Noceda-Rivera, DG. Mayen-Molina.* National Institute of Perinatology.

The fertility is the capacity to reproduce that have the human beings and the genetic factors play a fundamental paper. Of the 10 to 15% of the couples have some problem of the procreation. In the National Institute of Perinatology, the Clinic of Genetics of the Reproduction was formed as such from 1995 and to date it fulfills the requirements for the population's integral attention, being channeled the patients of the Clinic of Pregestational Risk or the Clinic of Biology of the Reproduction. To date a total of 9900 consultations, being in order of frecuency the factors for those couples have gone: recurrent abortion: 6069 (61.3%), previous descendence with birth defects: 1818 (18.36%), maternal age: 1308 (13.2%), sterility: 357 (3.6%). We have been carried out a total of 3900 karyotypes, being normals: 3708 (95.07%) and abnormals: 192 (4.92%). The form of boarding of the couple is shown, being simultaneous where the study plan is schematized, to the patients we dissipate the doubts and explain the procedures and diagnostic possibilities. According to the result we channeled to the suitable service, giving an integral attention. Should the need arise we also sent for their handling to other instances and we give to they the write material. Of this way their valuation is completed, being of extraordinary interest for the doctor in the systematic of diagnostic orientation.

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Twinning and assisted reproductive technologies in patients with Beckwith wiedemann syndrome. Data from the Spanish Overgrowth Syndrome Registry. *P. Lapunzina[1,2], L. Magano[2], A. Delicado[2], M.L. de Torres[1], M.A. Mori[1], F. Cabañas[3], L. Fernández[1], M. Palomares[1], I. Incera[2], P. Arias[2], I. López Pajares[1], R. Gracia[4].* 1) Dept Medical Genetics, Hospital Universitario La Paz, Madrid, Spain; 2) Dept Molecular Genetics, Hospital Universitario La Paz, Madrid, Spain; 3) Dept Neonatology, Hospital Universitario La Paz, Madrid, Spain; 4) Dept Pediatric Endocrinology, Hospital Universitario La Paz, Madrid, Spain.

Publications over the past years have seeded concern about the possibility of an increased incidence of rare genomic imprinting diseases in children born of assisted reproductive technologies (ART). Thus, increasing attention has recently focused on potential epigenetic disturbances resulting from embryo culture, somatic cell nuclear cloning and ART. On the other hand, monozygotic twinning occurs more frequently among Beckwith Wiedemann syndrome (BWS) patients than it does in the general population, and many of these twins are discordant for BWS. Three different studies pointed to an association between ART and the development of BWS. Seven patietns with BWS from USA, 6 from France, 7 from UK and 4 from other European countries have been born after ART so far. The Spanish Overgrowth Syndrome Registry has been established in 2003 aimed to record patients with overgrowth syndromes (OGS). It is located at the Hospital Universitario La Paz, in Madrid, Spain. All patients with an OGS (and in some cases their relatives) are included in a specific database. Nowadays, more than 30 Hospitals have been contributing to the Registry, and about 300 patients have been recorded. In addition, clinical photographs are requested. Written permission for both clinical photographs and biological samples are obtained. About 47 patients with complete data and diagnosis of BWS, 5 showed either twinning or had been born after ART. The table shows the main characteristics of these patients.

# Patient	Pregnancy	Twinning	The other twin	Molec. studies
1	Without ART	Double	No affected	11p UPD
2	ICSI	Double	No affected	ND
3	ICSI	Double	No affected	ND
4	ICSI	Double	No affected	Alt. Imprinting
5	ICSI	Single	-	Pending

Our data also confirmed previous reports in large series of patients with BWS. In these series an increase of twinning and children born after ART have been observed. These data also pointed out that epigenetic mechanisms are probably related to both the techniques of ART and the biological process of twinning.

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Chimerism in a phenotypic male with 46,XY/46XX and lack of spermatogenesis. *D. Wand, C. Gläser, C. Höfers, I. Hansmann.* Institut für Humangenetik und Medizinische Biologie, MLU Halle-Wittenberg, Halle (Saale), Germany.

Chimerism is being used to describe an organism with cells from two or more zygotes or their derivatives. True chimerism appears to be extremely rare being detected mainly by somatic effects of coexisting XX/XY cells. The few cases reported are hermaphrodites or of female phenotype. We report about an extremely rare case of a 35 year-old male with azoospermia and chimerism. He was referred with his female partner for genetic counselling preceding infertility treatment. He was born after an uneventful pregnancy to nonconsanguinous parents. He has one older clinically normal brother. At an age of 6 years penile hypospadia was corrected in addition to herniotomy, right. At the age of 14 years he was hospitalised due to mammary hyperplasia, orchiectomia left and orchidopexia right. Examination at an age of 24 years revealed hypergonadotropic hypogonadism and lack of any spermatogenesis. Testosteron substitution was initiated. Karyotyping of cultured lymphocytes revealed 46,XY and 46,XX in 66 % and 34 %, respectively of cells analysed. FISH with X and Y chromosome specific probes confirmed the results (68 % with XY and 32% with XX signals). For further investigation genotyping was performed by PCR for SRY and 40 microsatellite markers spread over 10 different chromosomes. Probands DNA from peripheral blood is positive for SRY and demonstrates heterozygosity for 2 alleles at all X chomosomal loci tested. Altogether 8 of the 35 autosomal loci tested revealed 3 alleles. At the remaining loci only 1 allele or 2 alleles were detected. The data observed are compatible with chimerism derived from 2 cell lines each containing an identical haploid genome from one parent and a different haploid genome from the other parent. A similar constitution has been reported for a 46,XX/46,XY hermaphrodite. Results from genotyping were consistent with a double paternal and single maternal genetic contribution (Giltay J et al. 1998). The most likely mechanism suggested i.e. a parthenogenetically dividing haploid ovum and fertilization of both resulting haploid and genetically identícal blastomeres by 2 sperm may hold true also to explain the genetic constitution of our case.

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Autosomal Dominant Coffin-Siris Syndrome With Premature Thelarche. *M. Flynn[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Coffin-Siris syndrome is a well-known MCA/MR syndrome [OMIM 135900]. Diagnostic criteria have evolved to include growth restriction, MR, absence of the terminal phalanges of the fifth finger, hypoplasia or absence of the fifth fingernails and toenails, hirsutism, and characteristic coarse facial features. Although typically AR, AD inheritance has also rarely been reported. We describe a mother and her two daughters who meet the clinical diagnostic criteria for Coffin-Siris syndrome. Both sisters had FTT, hypotonia, and dev delay. Multiple minor dysmorphic features consistent with Coffin-Siris syndrome were noted for both sibs. Sib 1 had a VSD, delayed bone age, premature thelarche, foreshortened halluces, hypoplastic fifth toenails, bilateral fifth finger clinodactyly, and rocker-bottom feet. She did not have adrenarche. RUS, ophthalmology exam, head MRI, HR chromosomes, subtelomeric FISH, and FISH 22 analyses were all normal. Sib 2 had PPS. She had right severe hearing loss and left mild conductive hearing loss. She had Tanner I genitalia and no thelarche. She had hypoplastic fifth toenails and shortened fifth toes bilaterally. RUS demonstrated an extra left renal pelvis with no evidence of hydronephrosis. Foot Xrays for Sib 1 noted absent distal phalanges of the fifth toes and hypoplastic middle phalanges of the fifth toes. Xrays for Sib 2 noted that the distal phalanges of the fifth toes had not yet ossified. Hand Xrays for Sib 1 noted hypoplastic distal phalanges of the fifth digits with absent epiphyses. The mother of these two sibs required special assistance in school. Her facial features and Xray findings are similar to her daughters. Hence, AD inheritance is the likely mode of inheritance in this family. Premature thelarche has previously been reported once in Coffin-Siris syndrome (Brunetti-Pierri et al., 2003). One of our patients is the second reported individual with premature thelarche, providing further evidence that it may be a clinical feature of the syndrome.

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EGR2 mutations: a natural history study of 10 patients and functional analysis of neuropathy associated alleles.

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We ascertained 10 patients with EGR2 mutations and examined the natural history of the disease by regarding progression and respiratory compromise [mean follow-up 17.5+/-10]. Three patients were from an AD, 3 from AR family and in 4 patients the sporadic neuropathy was associated with de novo mutations. Even the same mutations caused different phenotypes; e.g. heterozygous R359W caused CMT1, DSN and CHN in three different families. Respiratory compromise in the form of documented restrictive pulmonary disease was present in 3/7 (43 %) unrelated patients, in one case resulting in respiratory failure and death at 6 years. Cranial nerve findings were present in 60 % and involved the facial nerve, cranial nerve III, IX and XII. The progression of the disease was rapid, moderate or mild, depending upon the mutation. Interestingly, the toxic gain of function mutations resulted in moderate to severe progression, whereas the homozygous loss of function mutation in the AR family had minimal, if any, progression of the neuropathy. In vitro functional studies were performed, including transcription activity assays and localization studies utilizing constructs generated by in vitro mutagenesis. We did not find a correlation between mutations and outcome, thus it is extremely difficult to prognosticate patients with EGR2 mutations. However our study confirmed that respiratory compromise and cranial nerve dysfunction are commonly associated with EGR2 mutations and can be useful clinical findings in guiding molecular diagnostic.

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The confirmation of the diagnosis of CHARGE syndrome with the first Finnish adult patient. *S.J. Kivirikko, S. Ala-Mello.* Deparment of Clinical Genetics, Helsinki Univ. Centr. Hospital, Helsinki, Finland.

The acronym CHARGE refers to an association first described in 1979. With an estimated birth incidence of 1:12.000, this condition is a rather common cause of multiple congenital anomalies. The combination and the severity of the malformations can be very variable. The major anomalies include coloboma, heart malformation, choanal atresia, growth and/or mental retardation, genital anomalies, ear anomalies and/or deafness. Our patient was a 39-years-old woman without a specific diagnosis having several health problems. We report her natural history with photographs. Bilateral sensorineural hearing loss was detected at the age of 3 years. She has bilateral chorioideal coloboma, but the visual loss is not remarkable. She has renal agenesis on the right side, but no cardiac malformation. The right malformed ear has been operated in childhood, after which she has been able use the hearing aid. The most patients with CHARGE diagnosis have mutations involving CHD7 gene (chromodomain helicase DNA-binding protein-7) (Vissers et al Nature Genetic 36:955-957, 2004). After that it is more appropriate to speak about CHARGE syndrome than association. The confirmation of the clinical diagnosis of our patient was perfomed by detecting a pathogenic mutation 5982GA (W1994X) heterozygously in exon 30.

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Osteopathia Striata with Cranial Sclerosis: A clinical report of two Asian Indians. *S. Reddy.* Health Diagnostic Lab, Southfield, MI.

This clinical report describes two cases of Osteopathia Striata with Cranial Sclerosis (OS-CS) in two Asian Indians from Southern part of Indian subcontinent. A 25-year old man was referred for management of osteopathia striata with cranial sclerosis. The patient had multiple congenital abnormalities including cleft lip, hypertelorism, flat nasal bridge and prominent occipital bony protrusion. Radiographic evaluation revealed cranial sclerosis, longitudinal striations in the metaphyses of femur and tibia. Serum calcium, phosphorus and alkaline phosphatase were normal.Bilateral mixed hearing loss was present. The second patient was 31-year old woman presented with multiple congenital abnormalities such as cleft lip, cleft palate, hypertelorism and club feet. There was bilateral decreased hearing. She complained chronic lower extremity pain but there were no fractures. She had severe intellectual impairment.This syndrome is familial and is believed to be transmitted as an autosomal dominant trait but some authors suggested X-linked inheritance. Longitudinal striations of osteosclerosis in the long bones, disfigurement in the cranial and facial bones due to osteosclerosis were discussed.

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A new recessive connective tissue disorder with fleshy swelling of lips, lid and cheeks, macrocephaly, hyperextensible skin and severe scoliosis. *A. Verloes[1], A. Benmansour[2], G. Mortier[3], G.E. Pierard[4], M. Le Merrer[5].* 1) Clinical Genetics Unit and INSERM U676, Hosp Robert Debre, Paris, France; 2) Pediatrician, Oran, Algeria; 3) Dpt of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 4) Dpt of Dermatopathology, University Hospital Sart Tilman, Liege, Belgium; 5) Dpt of Genetics and INSERM U393, Hopital Necker Enfants Malades, Paris, France.

We report on three sibs born to first cousins Algerian parents, affected by the same progressive MCA syndrome. The hallmarks of this syndome are: 1) macrocephaly (+ 2 to + 3 SD), 2) progressive, fleshy swelling of the lip, cheeks and upper eyelids, 3) retrognathia, gothic palate and crowded teeth, 4) very sparse eyebrowses and mildly sparse, normally growing hair, 5) protruding, simple ears, 6) hyperextensible, non loose skin with easy bruisability, 7) severe, progressive scoliosis and 8) severe hyperlaxity of ankle joints and lesser hyperlaxity of small joints. Mental development was normal (1/3) or slightly delayed. One child had seizures. There was no bone dysplasia nor dysostosis, no bone fragility, no blue sclerae, no papyraceous scars, no valvular anomalies, no arachnodactyly. Skin biopsy showed alterations compatible with Ehlers-Danlos syndrome. This family appears to show a unique syndrome with Ehlers-Danlos-like dermatological features, facial dysmorphism, progressive swelling of the soft tissues of the face, and severe scoliosis, recessively inherited. To our best knowledge, this constellation of findings has not been previously reported.

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GENETIC AUTOPSY NORMATIVE DATA. *R. Lebel, J. Archie, J. Collins.* Greenwood Genetic Ctr, Greenwood, SC.

While the medical genetics community has long advocated the importance of autopsy to clarify the etiology of adverse pregnancy outcome, only a small number of geneticists participate directly in obtaining and interpreting prenatal growth data in the autopsy suite. Literature on the interpretation of weights and measurements is somewhat scattered and disparate; some reports organize data by gestational age, while others categorize by another weight or measurement. Worldwide literature on normative data (taken, insofar as can be determined, from non-dysmorphic individuals) was reviewed. Meta-analysis undertaken on crown-heel and crown-rump lengths; circumferences of the head, chest, and abdomen; hand and foot lengths; inner canthal, outer canthal, and inter-nipple distances; and weights of body, brain, thymus, heart, lungs, spleen, liver, kidneys, and adrenals. Despite the diversity of the populations studied, there is impressive concordance, such that meaningful and clinically useful regression curves could be generated in most cases. In other cases existing published regressions were used. The regression curves were incorporated into a computer program, aiding the interpretation of these anthropometric features by calculating percentiles and proportions of the expected mean, applicable to stillborn or neonatally deceased individuals. The program is currently used in a statewide fetal and neonatal autopsy service, adding greater precision to reports of findings. This improves the probability of accurate syndromic diagnoses, with greater consequent overall success in the autopsy effort. Thus it promotes the three major goals for such a service: (1) assisting in the familys grief resolution, (2) appreciating the etiology and therefore also the recurrence risk, and (3) adding to the body of knowledge of the epidemiology of genetic disease and pregnancy loss.

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Staged separation of craniopagus conjoined twins (CCT): Medical and neurodevelopmental status one year after separation. *R. Marion, J. Goodrich, D. Staffenberg.* Dept Pediatrics, Neurosurgery and Plastic Surgery, Childrens' Hosp at Montefiore, Bronx, NY.

Occurring in 1 in 10 million livebirths, craniopagus conjoined twinning is a rare, devastating anomaly. Literature review reveals that attempts to separate CCTs using a single surgical procedure have resulted in one or both twins either dying or suffering permanent neurologic damage. We describe a set of CCTs in whom separation was accomplished using a staged surgical approach and present an update on their condition 1 year after separation.

Born in the Philippines via c section in 4/02, the twins arrived at our center at 17 mos of age. MRI and MRA revealed that although scalp, skull, dura and cerebral venous return were shared, brains appeared to be separate. Multiple medical problems, including FTT, DD, severe hypertension in one twin, etc. were also noted; therapy to improve these was begun. Rather than attempt separation in a single surgical session, we chose to perform multiple smaller operations; during each, bridging vessels were divided in hopes that the twin in whom no venous return from CNS existed would generate such vessels from collaterals.

Over 10 months, 4 procedures were performed. The twins tolerated each procedure well and received PT, OT and ST between surgeries. Repeat MRA showed that a second plexus of veins had developed. Final separation was accomplished in early August 2004.

Nearly one year after separation, the boys are healthy, with height, weight and HC all in normal range. Medical problems have included hypertension in one twin (resolved after separation), OSA (treated with T & A), and serous OM (treated with M & T). Though they continue to exhibit speech delay, they are walking, riding tricycles, and showing no evidence of permanent neurologic deficit.

We describe the first case of intact survival of both members of a pair of CCT. The status of these twins one year after separation strongly suggests that staged separation is the optimum management for such twins.

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A de novo missense mutation in a critical homodomain of the POU3F4 gene caused X-linked male congenital profound sensorineural hearing impairment in a large Chinese family. *Q.J. Wang[1,2,4], Q.Z. Li[1,4], S.Q. Rao[3,4], Y.L. Zhao[1], H. Yuan[1], W.Y. Yang[1], D.Y. Han[1], Y. Shen[2].* 1) Department of Otolaryngology/ Head and Neck Surgery, Chinese PLA General Hospital, Beijing 100853, China; 2) Chinese National Human Genome Center, Beijing 100176, China; 3) Departments of Cardiovascular Medicine and Molecular Cardiology, the Cleveland Clinic Foundation, OH 44195, USA; 4) These authors contributed equally to this work.

It is known that about 5% of male congenital profound hearing impairment are inherited in X-linked inheritance, but its underlying molecular determinant remains to be identified. A large five-generation Chinese family with multiple familial cases of gender-specific congenital profound hearing impairment was identified and clinically evaluated. Model-based genetic linkage analyses were performed with the use of microsatellite polymorphisms to determine the locus for the sex-linked trait. Positional cloning and mutation analyses were performed with the family and unrelated population-based controls to establish molecular evidence that caused the specific X-linked inheritance pattern in the large Chinese family. Clinical investigations of the pedigree demonstrated the striking feature of the extremely high penetrance in the male members, but no penetrance in the female members. Linkage analyses mapped the disease to the chromosomal region Xq13.1-Xq23 (maximum X-linkage lod score, 3.27). Mutation screening of the candidate genes in the linkage region by direct sequencing revealed a de novo missense substitution (925T-->C) in POU3F4 gene, which leads to a substitution (Ser309Pro) of a serine for a proline residue at amino acid position 309. To further exclude the possibility that this mutation is a polymorphism, we conducted direct sequencing on 240 unrelated control subjects. None of the control subjects had the de novo mutation. In conclusion, multiple analysis approaches demonstrated that these disorders were caused by a founder mutation in POU3F4 gene. Our findings provided novel molecular evidence to implicate the pleiotropic role of POU3F4 gene in the development of hearing loss in humans.

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Cornelia de Lange syndrome or Goldberg-Shprintzen syndrome? From the clinical phenotype to the mutational analysis of NIPBL and KIAA1279 genes through the natural history. *M.L. Giovannucci Uzielli, M. Ottaviani, M. Levi, S. Toccafondi, L. Di Medio, S. Stagi, L. Carosi, S. Guarducci, U. Ricci, E. Lapi, L. Giunti.* Paediatrics Genetics Unit, University of FLorence, Florence, Florence, Italy.

In three of four children of consanguineous Italian parents, on the basis of the clinical phenotype only, we firstly posed a diagnostic hypothesis of Cornelia de Lange syndrome: especially significant where the microcephaly and the mental retardation in the three children, and the facial characteristics more expressed in the propositus, a 10 year old boy. The partially meaningful clinical patterns of the two sisters, and the presence of other discordant clinical aspects, kept nevertheless us hesitant on the definitive diagnosis. The natural history, observed in the propositus and in one of the two sisters, during more than ten years, offered us new useful diagnostic data: in particular, recurrent episodes of bowel pathology due to severe and progressive Hirshsprung disease, recently brought us to consider the diagnosis of Goldberg-Shprintzen syndrome, a rare AR disorder, characterized by microcephaly, mental retardation, Hirshsprung disease, and a peculiar face, in part superimposable in CdLS and GOSHS. We hope that the ongoing mutational analyses of NIPBL and KIAA1279 genes, may clarify the molecular basis of the clinical phenotype, to reach a final diagnosis of the disorder recurrent in this consanguineous family.

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Bladder exstrophy associated with congenital macrothrombocytopenia of Fechtner type - evidence for a common genetic cause? *B. Utsch[1], S. Karle[1], V. Schuster[2], H. Lenk[2], U. Jacobs[1], A. Kujat[3], H. Reutter[4], W. Rascher[1], J. Dötsch[1], J. Martignetti[5], M. Ludwig[6], R.B. Tröbs[7].* 1) Dept. of Pediatrics, University Erlangen/Nuremberg, Erlangen, Germany; 2) Dept. of Pediatrics, University of Leipzig, Leipzig, Germany; 3) Dept. of Human Genetics, University of Leipzig, Leipzig, Germany; 4) Dept. of Human Genetics, University of Bonn, Bonn, Germany; 5) Dept. of Human Genetics, Mount Sinai School of Medicine, New York, NY, USA; 6) Dept. of Clinical Biochemistry, University of Bonn, Bonn, Germany; 7) Dept. of Pediatric Surgery, University of Leipzig, Leipzig, Germany.

Bladder exstrophy is a rare urogenital malformation. Hitherto, no underlying gene defects are known but this group of malformations seems to be genetically heterogeneous. We report on a case of exstrophic bladder associated with congenital macrocytic thrombocytopenia. The parents are not consanguineous and family history was unremarkable. Laboratory results were normal except for macrothrombocytopenia of 10,000/l and platelet volume of 13.9 fl. An amniocentesis showed a normal karyogram. Hematological examinations revealed an unremarkable granulocytopoiesis and erythropoiesis. Since no leukocyte inclusions were found Epstein syndrome was suggested for which mutations in *MYH9* on chromosome 22 are known, and which are inherited in an autosomal-dominant manner. The locus for Opitz II syndrome for which urogenital malformations are reported is located next to the *MYH9* gene on 22q11.2 (distance ~12.2 Mb according to www.genome.ucsc.edu) but both loci did not show linkage to each other. FISH analyses with probes *TUPLE1* und *N25* detected no gross alteration. Direct sequencing of the *MYH9* gene yielded a heterozygous de-novo missense mutation (C287T) in the index patient resulting in an amino acid exchange S96L. The mechanism for the association described remains unclear since the reported mutation in *MYH9* was already described previously without a similar urogenital phenotype. Nevertheless, it can be due to unknown mechanism in the surrounding region or a co-inheritance of a neighboring mutated gene. Additional potential symptoms of Epstein syndrome could be still inapparent in our patient.

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Maternal Uniparental Disomy for the whole chromosome 15 in a newborn with classic Prader Willi syndrome phenotype, associated to a large interstitial deletion of chromosome 15q encompassing the entire Fibrillin-1 gene sequence. Molecular studies, and 3-years clinical follow-up. *M. Levi, M. Ottaviani, S. Toccafondi, L. Di Medio, S. Stagi, L. Carosi, S. Guarducci, I. Sani, U. Ricci, E. Lapi, L. Giunti, M.L. Giovannucci Uzielli.* Dept Paediatrics, Genetics Unit, University of Florence, Florence, Italy.

In a newborn male, with classic PWS clinical phenotype, we identified a UPDmat15: by using a battery of DNA polymorphic markers, to well define the mat(15)UPD, we identified an unexpected interstitial submicroscopic deletion 15q21 of one of the two maternal chromosomes 15, encompassing the entire Fibrillin-1 gene. MLPA-based analysis confirmed the complete absence of FBN1 gene. Maternal Uniparental Disomy (UPD) accounts for approximately 25% of PWS patients. A parent of origin specific methylation imprint has been demonstrated for the chromosome 15q11-13 region using methylation sensitive restriction enzymes, in all cases of PWS, characterized by deletion, UPD or imprinting mutation. Marfan syndrome is an Autosomal Dominant multi-system connective tissue disorder, due to mutations in the Fibrillin-1 (FBN1) gene. More than 200 different mutations were reported in the 235 kb, 65 exon-containing gene, on 15q21. Only few multi-exon FBN-1 deletions were discovered. We report the first case of entire deletion of FBN-1 gene: we found no other cases described in the literature, but we heard of a subject with complete heterozygous deletion of FBN-1 gene and clinical Marfan phenotype. Our patient is now 3-years old: his staturo-ponderal development is between the 25 and the 50 centile. The clinical phenotype is striking suggestive of PWS, no clinical patterns of Marfan syndrome are until now present. It could be interesting to consider how features of PWS might make Marfan features less obvious, and viceversa. No significant ultrastructural variation of the skin and subcutaneous tissue. No cardiovascular, ocular or orthopaedic anomalies. This unique case of complete heterozygous deletion of FBN1 gene may expand the knowledge of mutational mechanisms and genotype/phenotype correlation of fibrillinopathies.

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Associated malformations in children with limb reduction defects. *B. Dott, Y. Alembik, M.P. Roth, C. Stoll.* Medical Genetics,Faculte de Medecine,Strasbourg,France.

Infants with limb reduction defects(LRD)often have other associated congenital defects.The purpose of this investigation was to assess the prevalence and the types of associated malformations in a defined population.The prevalence and types of associated malformations in infants with LRD were collected in all livebirths,stillbirths and terminations of pregnancy between 1979 and 2002 in 320,810 consecutive births in the area covered by our population based registry of congenital anomalies.Of the 246 LRD infants born during this period,58.1% had associated malformations.Associated malformations were more frequent in infants who had upper limb reduction defect(63.2%) than in infants with lower limb reduction defects(48.4%).Malformations in the cardiac system and in the central nervous system were the most common other malformations,15.2% and 10.6% of the associated anomalies,respectively,followed by anomalies in the genital system(10.1%),in the renal system(6.8%),and in the digestive system(6.3%).There were 16(6.5%)cases with chromosomal abnormalities,including 8 trisomies 18,and 2 22 q 11 deletion, and 56(22.8%) nonchromosomal dysmorphic syndromes.There were no predominant dysmorphic syndromes,but VA(C)TER(L) association.However numerous dysmorphic syndromes were registered including,among them,the following : EEC,OFD,Klippel-Trenaunay-Weber,OAVS,CHARGE,Townes Brocks,Moebius,Du Pan, SLO,hypoglossia-hypodactyly,amniotic band,De Lange,Rubinstein-Taybi, Fanconi,TAR,Roberts,Holt-Oram,and fetal diethylstilbestrol.Seventy one(28.8 %)of the cases were multiply,non syndromic,non chromosomal malformed infants.Prenatal diagnosis was performed in 48.8 % of dysmorphic syndromes with LRD,whereas prenatal ultrasonographic detection was only 23.9 % in cases with isolated LRD.The overall prevalence of associated malformations,which was more than one in twoinfants,emphasizes the need for a thorough investigation of infants with LRD.A routine screening for other malformations especially cardiac,central nervous system,urogenital system,facial clefts,and digestive system may need to be considered in infants and in fetuses with LRD.

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Multiple congenital anomalies in a surviving donor twin of an acardiac twin pregnancy; further support for a vascular etiology of oculo-auriculo-vertebral spectrum. *R. Smith[1], S. Ellingwood[1], D. Dressel[2], R. Chard[3], J. Wax[3], M. Pinette[3].* 1) Div Genetics, Dept. of Pediatrics; 2) Dept. of Pathology; 3) Div. of MFM, Dept. of OB/Gyn, Maine Medical Center, Portland, ME.

We report a 4 mo. old male, former donor twin of an acardic twin pregnancy, whose various anomalies fit the oculo-auriculo-vertebral (OAV) spectrum. He was the product of a 32 6/7 wk pregnancy born to a 20 y.o. G2P0 mother. Chromosomes were 46, XY and 22q11.2 FISH deletion studies were normal. The pregnancy was followed conservatively for TRAP sequence diagnosed at 16 wks gestation. The infant was born by elective c-section prior to signs of heart failure. His birth weight was 1.66 kg. Congenital anomalies noted at birth included; ileal atresia, right sided microtia with atretic external auditory canal, right sided hemifacial microsomia, cutis aplasia on the scalp, abnormal cervical and thoracic vertebrae with several fused ribs on the right, small muscular VSD and bicuspid aortic valve, hypoplasia of the distal phalanx of the right thumb, and left renal pelviectasis. His acardiac twin had a classic appearance with no true head, but developed trunk and limbs, with multiple internal and external anomalies. The placenta was monochorionic, monoamnionic with a single cord stalk which bifurcated 1.5 cm above its insertion. Each cord had two vessels. A complex true knot was present 2.0 cm. above the bifurcation.

The etiology of OAV spectrum is felt to be heterogeneous with support for both environmental and genetic influences. Abnormal vascular flow has experimentally been shown to cause hemifacial microsomia, and has been suggested to be a factor in the OAV spectrum. We postulate that abnormal blood flow existed in this pregnancy as demonstrated by the discordant acardiac twin which has a known vascular pathogenesis, by placental morphology, and by the additional finding of ileal atresia and cutis aplasia in this infant. This case argues for a placental anastamosis mediated vascular pathogenesis as the cause of the multiple anomalies seen in this case and supports the hypothesis of a vascular etiology in OAV spectrum.

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Changes in the prevalence of at birth congenital malformations in Belarus in view of the Chernobyl accident. *G.I. Lazjuk, O.I. Zatsepin, R.D. Khmel, I.L. Babicheva.* National Research Institute of Hereditary and Congenital Diseases, Minsk, Belarus.

In Belarus, since 1979 on the bases of Institute of Hereditary and Congenital Diseases a republican registry of congenital malformations (CM) has been functioning. Data over 40 thousand fetuses and newborns with congenital malformations was recorded by the beginning of the year 2005 in the republican registry of CM. A Long-term research conducted in the field of possible teratogenic and genetic consequences of the Chernobyl accident gives possibility to draw the following conclusions: I.Since the middle of eighties a steady increase of CM prevalence is observed in Belarus, found similar in contaminated and control areas. II.From 1987 to 1989, a significant access of the prevalence at birth of mandatory registered (MR) CM was observed in the regions, ascribed to the strict radiological control area (SRCA) with 137Cs soil contamination exceeding 15 Ci/km2, as compared to the control. Relative risk (RR) for MRCM was found RR1987-1989 = 1.57 with 95% confidence interval being = 1.29;1.89 III.No direct teratogenic effect of the impact of Chernobyl NPP fallouts on fetus was registered. CM of central nervous system might be the only one possible exception. An increased prevalence of at birth of anencephaly (0.8‰) was observed in the regions of SRCA in 1987, significantly acceding the control values (0.2‰) (RR1987 = 4.49; 95%CI = [1.12; 17.95]). Moreover, more than twofold increase of the percentage of head circumference deficiency was registered among the newborns (n=912), born in the second half of 1986 and first half of 1987 whose intrauterine development took place in the mostly contaminated areas of Gomel region, as compared to the control group (n=1121). IV.Maximal monthly cluster of Downs syndrome (31 cases registered vs. 14 expected) with the highest increase observed in Gomel region; as well as an increased CM prevalence with high contribution of de novo mutations (multiple CM, reduction defects of limbs and polydactyly), registered in SRCA within the first 3 years after the accident, might be an indicator of indirect teratogenic effect due to gametic mutations.

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Maternal MTHFR genotype contributes to the risk of non-syndromic cleft lip and palate. *N.H. Elcioglu[1], A.S. Yusufagiç[1], O. Celebiler[2], M.U. Erdim[2], R. Bircan[3], C. Erzik[3].* 1) Department of Pediatric Genetics, Marmara University Medical School, Istanbul, Turkey; 2) Department of Plastic Surgery, Marmara University Medical School, Istanbul, Turkey; 3) Department of Medical Biology, Marmara University Medical School, Istanbul, Turkey.

The pathogenesis of orofacial cleft lip with or without cleft palate (CL/P) is complex with the interaction of various genetic and environmental factors. Recently MTHFR functional polymorphisms were found to increase the risk of this common malformation, although this finding is still debated. In order to determine whether MTHFR variants are risk factors for nonsyndromic CL/P in Turkish population we carried out a case- parents triad study. 56 non-syndromic CL/P patients, their parents were genotyped for the 677C>T (p.Ala222Val) and 1298A>C (p.Glu429Ala) polymorphism in the MTHFR gene. Genotype and allel frequencies were compared with 2 control groups from the same population used in previous studies. Additionally the transmission of variant alleles from parent to the affected child were analyzed with the transmission disequilibrium test (TDT) in 46 trios. As a result TDT analysis showed no significant distortion in both allele transmission. There was also no difference for MTHFR 677C>T mutation frequencies between CL/P cases and their fathers compared to both control groups. But the association studies revealed significant differences in allele frequencies between mothers of CL/P patients and the controls. Mother carrying 677C>T homozygote genotype had 3 fold higher risk for having a CL/P child when compared to controls (**OR: 3.14, p: 0.03). The 'combined heterozygosity' (677C>T /1298A>C) genotype which was observed in % 28 of the mothers, remained insignificantly compared to the controls (OR: 2.27, p:0.07). This work supports the hypothesis that a lower MTHFR enzyme activity in pregnant women, commonly related to the homozygote MTHFR 677C>T polymorphism, could be responsible for a higher risk of having CL/P affected offspring. Therefore these mothers should treated with higher dose of Folic acid during the periconceptional period, rather than the recommended low dosage for the general population prevention.

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NSD1 polymorphic DNA markers for microdeletion screening in patients with Sotos syndrome. *I.. Kondo[1,3], H. Yamagata[1], Y. Tabara[1], T. Miki[2], F. Yamaguchi[3], K. Kuwajima[3].* 1) Dept Medical Genetics, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Geriatric Genetics, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept Pediatr. Ibaraki Prefectural Handicapped Children's Center, Ibaraki, Japan.

Sotos syndrome (SoS: OMIM 1175509 is an overgrowth syndrome associated with multiple congenital anomalis and developmental delay. Diagnostic characteristics are specific facial features (prominent forehead, receding hairline, hypertelorism, downward slanting palpebral fissures, small nose and prominent jaw) in addition to large head circumference and advanced bone age. Patients with SoS had either a common microdeletions including NSD1 gene or point mutations in NSD1 gene. The microdeletions were identified in majority SoS patients in Japna, but point mutations were common in non-Japanese patients with SoS. However, a half of patients with clinical features of SoS had not any mutations in NSD1 gene, even after mutation screening by direct sequencig and FISH analysis. We have developed an easy screening method for microdeletions in NSD1 gene using PCR approach. Two polymorphic DNA markers in introns 3 and 17 in NSD1 gene were identified and only 12 in 257 individuals were homozygous for the intron 3 polymorphic marker in a Japanese population, suggesting that a heterozygosity for this marker was 95.3% in Japanese population. In addition, five polymorphic DNA markers in exons 5 and 23 of NSD1 gene were highly heterogeneous, but these markers were in complete linkage disequilibrium in Japanese population. Using these DNA markers in NSD1 gene, three patients with SoS were homozygous for the polymorphic marker in intron 3 of NSD1 gene and microdeletion was confirmed based on parental genotypes, and two patients with SoS who were heterozygous for these marker had missense mutations in NSD1 gene after DNA direct sequencing in NSD1 gene.

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Genetic counselling for early initiation of renoprotection in familial juvenile hyperuricemic nephropathy. *C. JACQUOT[1], D.P. GERMAIN[2].* 1) Nephrology, Hopital Europeen G. Pompidou, Paris, France; 2) Genetics, Hopital Europeen G. Pompidou, Paris, France.

Background : Familial juvenile hyperuricemic nephropathy (FJHN, OMIM 162000) is an autosomal-dominant disorder characterized by decreased urate excretion and the development of progressive interstitial nephritis leading to chronic renal failure. A locus for FJHN has been identified on chromosome 16p12 close to the MCKD2 locus, which is responsible for one type of medullary cystic kidney disease (MCKD2). UMOD, the gene encoding the uromodulin protein (also known as Tamm-Horsfall protein), maps within the FJHN/MCKD2 critical region and mutations in UMOD have recently been reported in families with FJHN/MCKD2, which are allelic disorders.

Case report : We describe a new family in which a 51-year-old male with hyperuricemia and renal insufficiency (serum creatinine 385 mol/L) was diagnosed with FJHN. His 21-year-old clinically asymptomatic son was found, through pedigree analysis, to have hyperuricemia and increased serum creatinine (148 mol/L), while his two daughters, with yet normal uricemia and serum creatinine levels, are awaiting molecular diagnosis. Renoprotection with allopurinol and angiotensin converting enzyme inhibitors was started in the probands asymptomatic son, in an attempt to avoid gout episodes and prevent progression of kidney failure. Interestingly, the probands parents are both healthy at 75, raising the hypothesis of a de novo mutation in the proband. They are currently being investigated for the presence or absence of the mutation in the UMOD gene.

Discussion : Thirty-one mutations associated with MCKD2 and FJHN in 205 patients have been described in the UMOD gene, with a cluster in exons 4 and 5. FJHN might be more frequent than previously thought. Sequencing of exons 4 and 5 of the UMOD gene is an easy way to confirm the diagnosis. Genetic counselling is key for the early onset of renoprotection in affected individuals in order to prevent hyperuricemia and progression of kidney insufficiency.

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VLA-4 gene polymorphisms in Italian patients with Multiple Sclerosis. *V. Andreoli, F. Condino, M. Liguori, I. Manna, A. La Russa, R. Cittadella.* Institute of Neurological Sciences,National Research Council, Pianolago di Mangone, Cosenza, Italy.

Multiple sclerosis (MS) is the most common human demyelinating disease of the central nervous system (CNS), caused by an interplay of environmental and genetic factors. Very late antigen 4 (VLA-4) is a member of the integrin family and it is considered to be a key adhesion molecule in immune responses. On the basis of its role, it is expected that VLA-4 inhibitors would have anti-inflammatory activity because blocking of this integrin has the potential to inhibit the process that play important roles in inflammation. Recently 4 integrin antagonisms are currently being evalutated as therapeutic agents in chronic inflammatory diseases, like MS. We hypothesized that, because the T-cell activation drives a conformational change that alters the VLA-4 molecule from a low-affinity to high-affinity state with its ligands, the polymorphic 4-subunit of VLA-4 gene may represent a good target for association studies in MS and could be involved as candidate for drug resistance to the disease. To address these findings, this study focused on two genetic polymorphisms of the 4 -subunit: a single point mutation at position 3061 producing an arginine (CGG) to glutamine (CAG) transversion at amino acid position 844, and a C to A transversion at position 269 in the promoter region of exon 1. However, we do not have in literature objective data on the frequency of this variants in MS. We investigated the association of two single nucleotide polymorphisms at position 269 in the promoter region of exon 1 and 3061 in the exon 24 of the VLA-4 gene, through a case-control study involving 280 Italian patients from southern Italy with definite MS, and 255 age and sex-matched healthy controls from the same geographical area. Our results showed no significant differences in the allele and genotype distribution of the VLA-4 polymorphisms between MS patients and controls suggesting that, despite a biological plausibility, this VLA-4 gene polymorphisms are not significantly associated with MS in Italian patients.

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Association of Variants in the CD244 Gene with Type 2 Diabetes in Pima Indians. *Y. Guo[1], V. Ossowski[1], J. Wolford[2], R.L. Hanson[1], S. Kobes[1], C. Bogardus[1], M. Prochazka[1].* 1) NIDDK/PECRB, NIH, Phoenix, AZ; 2) TGen,Phoenix,AZ.

A prior genome-wide linkage scan in Pima Indians indicated a type 2 diabetes mellitus (T2DM, onset age under 45 years) susceptibility locus on chromosome 1q21-q23. To narrow the location of the putative underlying mutation, we are analyzing single nucleotide polymorphisms (SNPs) across this region in over 1000 Pima Indians from the original linkage study. So far, linkage disequilibrium mapping showed several distinct areas associated with T2DM. One such region spans the CD244 gene which codes for a transmembrane receptor expressed in the immune system. Because abnormalities of the immune system have been recently implicated in the pathogenesis of T2DM, we investigated CD244 as a candidate gene. Sequencing of the 9 exons, exon-intron boundaries, 5' and 3' un-translated regions, and 1.6 kb of the 5' putative promoter of CD244 in 24 diabetic and 23 non-diabetic Pima Indians identified 9 variants, with a minor allele frequency between 0.08 and 0.20. The 3'UTR SNP rs485618 (G/A substitution) was associated with diabetes onset-age under 45 years (P=0.006, OR=3.19, 95%CI=1.40-7.28, additive model; P=0.008, OR=3.28, 95%CI=1.37-7.84 dominant model) after adjusting for sex, nuclear family membership and Pima heritage. The risk allele A had a frequency of 0.08. In a subgroup of 250 non-diabetic full-heritage Pimas who have undergone detailed metabolic testing including measurements of body composition, oral glucose tolerance, and hyperinsulinemic-euglycemic clamp, the risk allele A was marginally associated with lower glucose disposal rates (P=0.06, dominant model) after adjusting for age, sex, family membership, percentage of body fat. Although we did not detect any obvious functional variants (e.g. in the coding sequences, splice sites), the observed associations may reflect an effect of a variant on the expression of CD244, or could be markers for a nearby causative mutation. A CD244-/- knockout mouse was recently developed by a separate group, and we plan to investigate these mice for a potential effect of CD244 deficiency on the regulation of glucose metabolism.

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High prevalence of brachymesophalangia-V in an endogamous population from eastern Nepal. *K.D. Williams[1], J. Blangero[2], D.L. Duren[1], C.R. Cottom[1], S. Lawrence[1], T. Dyer[2], B. Jha[3], J. Subedi[4], S. Williams-Blangero[2], B. Towne[1].*
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Brachymesophalangia-V is a skeletal anomaly characterized by a short and broad middle phalanx in the fifth digit. It occurs frequently in individuals with certain genetic disorders (e.g., Down Syndrome), but is also observed in otherwise normal individuals. Brachymesophalangia-V appears to be an autosomal dominant trait, and studies suggest that it may be caused by mutations in the GDF5 gene on chromosome 20q. Population prevalence rates are generally very low (< 1%), but we have recently discovered an unusually high frequency of brachymesophalangia-V among children participating in the Jiri Growth Study, a genetic epidemiological study of child health conducted in the endogamous Jirel ethnic group of eastern Nepal. The Jirel population numbers approximately 5000, most of who are in one very large extended pedigree. A hand-wrist x-ray is taken annually of each child in the Jiri Growth Study to assess their skeletal development. X-rays of 1,310 Jirel children (645 boys; 665 girls) were examined for presence or absence of brachymesophalangia-V; 75 cases were observed in boys (11.6%) and 62 cases were observed in girls (9.3%), for a combined prevalence rate of 10.5%. As an initial step toward elucidating the genetic architecture of brachymesophalangia-V in the Jirel population, we used a variance components-based method for pedigree data (SOLAR; Almasy and Blangero, 1998) to estimate its heritability. The additive genetic heritability of brachymesophalangia-V is significant ($h^2 = 0.32$, $p = 0.004$). Future work will include collecting phenotypic data from older relatives of Jirel children who have brachymesophalangia-V to confirm its mode of inheritance, conducting linkage analyses to identify chromosomal regions harboring genes responsible for brachymesophalangia-V, and examining variations in candidate genes such as GDF5. Supported by NIH grants HD40377, AI37091, AI44406, and MH59490.

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Analysis of a cohort and case/control study for congenital malformations in 20.000 births of 8 Colombian hospitals. *N. Garcia, F. Gil, F. Suarez, I. Zarante.* Intituto de Genetica Humana, Pontificia Universidad Javeria, Bogota D.C., Distrito Capita, Colombia.

Objective: To determine the frequency of congenital malformations in 8 Colombian hospitals from June of 2001 to December of 2003 and to describe any association with prenatal risk factors. Method: A study with operating modality was carried out, case-control nested to a cohort during the period of the 1º of June of 2001 to the 31th of December of the 2003. All intrahospitalary births were considered; alive of any weight and stillborn over 500 g occurred in the room of labours of each hospital participating in ECLAMC-VIDEMCO project (Latin-American collaborative study of congenital malformations - Colombian study of congenital malformations) . For each malformed patient a form case was filled out and that of the respective control excepting the malformed stillborn which do not have control. Results: The total number of births was 20 485, 20 236 they were live birth and 249 stillborn. The proportion of congenital anomalies was of 3.50% (719). The 3.5% of the livebirth presented malformations in the group of stillborn they presented them in 13%. The associated factors that were found significantly to the presence of malformations were: consumption of alcohol (OR: 2.1 (1.28.3.5) 95% CI, antecedent of abortion (OR: 1.27 (1.02-1.58) 95% CI (1.28.3.5) and the vaginosis, OR: 1.46. (1.20-1.90) 95% CI. Conclusion: This initial approximation provides data on type and frequency of congenital malformations and prenatal risk factors in Colombia.

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Multiple voluntary articular subluxations in a boy with congenital onychodysplasia of the index fingers (Iso-Kikuchi syndrome): Two independent mendelian events? *L.O. Barajas-Barajas[1,2], C. García-García[1], J.R. Corona-Rivera[2].* 1) Servicio de Génética, Dirección de Rehabilitación, Sistema DIF Jalisco, Guadalajara, Jalisco, México; 2) Instituto de Genética Humana Dr. Enrique Corona Rivera, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

Benign joint hypermobility can be observed in about 9% of normal population and is a cardinal feature in more severe disorders, including Ehlers-Danlos syndrome, osteogenesis imperfecta and Marfan syndrome. Voluntary or habitual subluxation has been described for the hip and shoulder articulations. We present a boy with multiple voluntary articular subluxations who also showed congenital onychodysplasia of the index fingers (COIF), also named Iso-Kikuchi syndrome. The propositus, was born from a 21-year-old G1 healthy mother after a term pregnancy. Birth weight was 2850 g, birth length was 50 cm, and Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. Psychomotor development was normal. Examination at 3 years of age showed normal weight, length and OFC, dolichocephaly, small facies, epicanthal folds, down-slanting palpebral fissures, small chin, abnormal ears, narrow shoulders, genu recurvatum, skin syndactyly between second to third toe fingers, flat feet, and hyperelastic skin. He presented voluntary subluxations at the shoulder, elbow, hip, and fifth metacarpophalangeal articulations. Both index fingernails showed polyonychia (split rudimental). The remaining fingernails and toenails were normal. Skin biopsy reported increased elastic fibers. A 23-year-old father was referred with scapular hypermobility. Our patient fulfills the criteria for diagnosis of COIF. However, to the best our knowledge, observed articular findings has not been previously described in patients with COIF. It remains unclear if multiple voluntary articular subluxations in this case are related to COIF or are fortuitous probably mendelian associations.

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13 years follow up of patient with Schwartz-Jampel Syndrome. *M. Padilla R, L. Figuera V, C. Garcia G.* Genetics Division, CIBO, IMSS, Guadalajara, Jalisco, Mexico.

The 23 years old proposita was the product of the second uncomplicated pregnancy; from non-consanguineous and health parents (father and mother of 44 and 41 years respectively). She has been followed by our department since age of 4 years to date with diagnosis of Schwartz-Jampel syndrome (SJS). Normal mental development and no positive familiar history. The symptoms started at age of 2 years with severe retrognathia, bilateral eye blepharofimosis and at age of three she initiated facial deformation. To attract attention the off bone affectation, because it was publish who syndrome atypical form. The characteristics facials and abnormality dental be characteristics syndrome, but not abnormality bone. Exploration Normocephalic, normal implantation of hair line, large forehead, to level facies, lower implantation and posterior rotation ear, blepharophimosis, contracture of the facial muscles, thorax asymmetric with right to predominate, short and clinodactyly of 5th fingers. To exploration Intraoral to observe tooth bad position, retention tooth (2nd and 3rd lower molars), vestibularitation incisive, posterior cross bite, increase over bite, gingivitis, mouth respiration, microstomie, temporomandibular joint stiffness, microretrognathie. Electromyography showed miotony, metabolic screen show to positive mucopolisacaridosis and positive tiosulphate. Patient received application botulinic toxin, to leave it for application painful. SJS, (OMIM 255800) is a rare autosomal recessive disorder characterized by the presence of myotonia with a mask-like face and skeletal dysplasia with growth retardation. Although rare, SJS has been divided into two types defined by the age at manifestation of the symptoms. The clinical variability observed in SJS could reflect heterogeneity in the molecular basis of this condition. Linkage of SJS to human chromosome 1p34-p36.1 has been shown in families of different ethnic origins, suggesting that SJS results from mutations at a single locus, witch transcribe the protein heparan proteoglicain sulphate 2 (perlacan) (hspg2). However, all the families used for the published studies presented with the condition during infancy or early childhood.

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Molecular analysis of NIPBL gene in 40 Italian patients affected by Cornelia de Lange syndrome with wide phenotypic spectrum. *A. Selicorni[1], S. Russo[3], M. Bottigelli[1], D. Milani[1], A. Bentivegna[2], C. Cavalleri[3], C. Gervasini[2], G. Scarano[6], L. Memo[4], P. Castronovo[2], M. Masciadri[3], M.T. Di Vizia[7], C. Sforzini[8], E. Tarantino[5], L. Larizza[2].* 1) Pediatrics, University of Milan, Milan, Milani, Italy; 2) Medical genetics, Milan; 3) Ist Auxologico Italiano, Milano; 4) Genetica Clinica Treviso; 5) Genetica Clinica Pisa; 6) Genetica Medica Benevento; 7) Istituto Gaslini Genova; 8) Ospedale Valduce Como.

Cornelia de Lange syndrome (CdLS) is a MCA/MR disease characterized by pre/post natal growth retardation, facial dysmorphisms, mental retardation, hirsutism, small hands and feet or limb reduction defects. Occasional major malformations and specific medical complications are described too. Recently heterozygous mutations were reported in the previously uncharacterised gene, NIPBL, mapping in 5p13.1 and encoding a delangin protein. Mutation rate detected within CdLS patients ranges from 20 to 47%. DHPLC and direct sequencing of the 46 coding exons of NIPBL were carried on in a cohort of 40 patients with a medical diagnosis of CdLS and a wide variability in the expession of the phenotype. Every patient has been characterized as regard growth, psychomotor development, limb morphology, presence of major malformations and medical complications. Mutations were found in 15/40 patients, with a percentage of 37,5%.We found 14 sequence alterations, including 8 truncating, one in-frame deletion, 4 splice site and one missense, identified in 15 patients. Out of the 8 truncating mutations, two are stop codon, R797X and R2470X, the latter of novel description, while the remaining are never reported frameshift alterations, (459_460delC,2371_2372delC,2738_2743insTCACC, 2914_2915delA,7876_7877insA) with the exception of 2479_2481delAG described in two patients and recorded twice in our cohort. Correlating the genotype results to the phenotype we have found a relevant incidence of limb malformations in the mutated subjects (6 on 10 of the analized patients) while no clear correlation was demonstrated with severity of mental and growth retardation. We thank Fondazione Mariani and Associazione Italiana Sindrome di Cornelia de Lange for the collaboration and the support.

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Five novel mutations in the FGD1 gene in Aarskog-Scott syndrome. *T. Kaname[1], K. Yanagi[1], Y. Chinen[2], N. Okamoto[3], K. Kurosawa[4], M. Tsukahara[5], M. Yamada[6], T. Kondoh[7], K. Naritomi[1].* 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) Dept Pediatrics, Univ Ryukyus, Nishihara, Japan; 3) Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; 4) Clinical Research Institute, Kanagawa Children's Medical Center (KCMC), Yokohama, Japan; 5) Faculty of Health Science, Yamaguchi University School of Medicine, Ube, Japan; 6) Rihabilitation Center for Disabled Children, Kumamoto, Japan; 7) Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Faciogenital dysplasia 1 (FGD1) gene was identified as a responsible gene for Aarskog-Scott syndrome (AAS). The FGD1 mutations are found in approximately 20% of AAS patients and no genotype/phenotype correlation has been found. We found five novel mutations of the FGD1 gene in 9 Japanese patients with AAS. Of 9 patients, 4 patients are familial AAS (two families) and 5 patients are sporadic, and two sporadic patients do not have mutations in the FGD1 gene. There are two missense mutations in exon 6 and exon 11, one nonsense mutation in exon 15, and two point mutations at splice junction (exon 6 and exon 14). The mutations are different in each patient except in the same family. All mutations are suspected to reduce the FGD1 function. By comparison of clinical feature in AAS patients between with and without FGD1 mutation, it was suggested that a joint hyperextension, which includes hypertension of the proximal interphalangeal joints and flexion of the distal interphalangeal joints would be one of the typical feature of AAS with FGD1 mutation.

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Neuroblastoma in a patient with Noonan syndrome: Possible genotype/phenotype correlations in *PTPN11*. *A. Kwan, C.J. Twist, M. Manning.* Dept Pediatrics, Stanford Univ Med Sch, Stanford, CA.

Noonan syndrome (NS) is a multiple congenital anomaly syndrome characterized by short stature, webbed neck, dysmorphic facial features, pectus abnormalities, congenital heart defects, cryptorchidism, coagulation defects, and developmental delay. An increased association between NS and certain malignancies, such as juvenile myelomonocytic leukemia (JMML) and neuroblastoma, has been reported. Mutations in *PTPN11*, the gene encoding the protein tyrosine phosphatase Shp-2, have been identified in ~50% of NS patients. Genotype/phenotype correlations show that specific germline mutations in *PTPN11* are associated with the development of JMML in NS. Somatic *PTPN11* mutations have also been reported in isolated neuroblastoma. We report a patient with NS and a *PTPN11* mutation who also has neuroblastoma and discuss the importance of developing further genotype/phenotype correlations.Our patient is a 2 year 3 month old female referred for dysmorphic features, infantile scoliosis and developmental delay. Physical exam revealed a broad forehead, hypertelorism, downslanting palpebral fissures, epicanthal folds, low-set, posteriorly rotated ears, widely-spaced, inverted nipples, a low posterior hairline and marked scoliosis. Spinal MRI identified bilateral thoracic paraspinal and periaortic masses and a separate tumor of the left adrenal gland, consistent with stage 4 disease. Biopsy of the primary tumor confirmed the diagnosis of neuroblastoma without *MYCN* gene amplification. Constitutional chromosome analysis at the >550 band level and subtelomeric probes were normal. *PTPN11* mutation analysis revealed a G to T transversion in exon 3, resulting in a tyrosine to aspartate substitution at amino acid 62. Four patients have been described with NS and neuroblastoma. One had a *PTPN11* mutation in exon 13 and another had a tyrosine to cysteine substitution in the same residue affected in our patient. We report the third patient with NS and neuroblastoma who has a *PTPN11* mutation. This association suggests that further genotype/phenotype correlations are necessary and may aid in screening and management for NS patients who might be at risk for developing neuroblastoma.

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Morphologic characteristics in hemizygous Fabry disease patients. *M. Ries[1], D.F. Moore[2], C. Robinson[1], C. Tifft[3], K. Rosenbaum[3], R.O. Brady[1], R. Schiffmann[1], D. Krasnewich[4].* 1) Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 2) Section of Neurology, Department of Internal Medicine, University of Manitoba, Canada; 3) Division of Genetics, Metabolism, and Center for Prenatal Evaluation, Children's National Medical Center, Washington, DC; 4) Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Objectives: 1) To characterize morphometric characteristics in hemizygous patients with Fabry disease (FD). 2) To evaluate internal consistency of the proposed Fabry dysmorphology scale and the inter-rater-variability.

Design: Cross-sectional dysmorphology assessment by a panel of three independent clinical geneticists.

Participants: N=38, mean age 3810.8 years (range: 10-60).

Results: We identified periorbital fullness, prominent lobules of the ears, bushy eyebrows, recessed forehead, acute nasal angle, broad fingertips, generous nose/bulbous nasal tip, prominent supraorbital ridges, shallow midface, full lips, prominent nasal bridge, broad alar base, short fingers, prominent superficial vessels of hands and feet, coarse features, posteriorly rotated ears, and 5th digit brachydactyly. Crohnbachs alpha was 0.68. After principal component analysis (PCA) the initial 25-feature set was statistically reduced to the number of independent features supported by the data set. The statistical dimensionality of the data set determined by PCA suggested a core of 10 features. Lights kappa for n-observer inter-rater-variability was 0.38 while Cohens kappa allowing pair-wise rater comparison varied between 0.20 - 0.53.

Conclusions: Patients with FD have common characteristic morphological features of the face, trunk, and extremities. Some of these features are subtle as documented by the inter-rater-variability. Awareness of these features may facilitate the diagnosis of patients with FD and identification of affected family members.

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Quantitative changes in the dermatoglyphic phenotype of Nail Patella Syndrome (NPS). *C.A. Brandon[1], N.M. Scott[2], K. Neiswanger[1], S.M. Weinberg[1], K.M. Bardi[1], B.S. Maher[1], A.L. Towers[3], M.L. Marazita[1].* 1) Ctr for Craniofacial and Dental Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 3) Div Geriatric Med, Univ Pittsburgh, Pittsburgh, PA.

NPS is a rare genetic developmental disorder (22/1,000,000) caused by mutations in the LMX1B gene on 9q34, which is responsible for dorsoventral patterning and limb development. Multiple hand and nail anomalies are seen in individuals with NPS, including nail dysplasia with a gradient of severity across the hand, an increase in arch patterns and an increase in extralimital triradii. Dermatoglyphic data was ascertained from 29 unrelated NPS cases, from unaffected family members (UFM, n=7), and controls from the literature (n=720). Total (TRC) and absolute ridge counts (ARC), stratified by hand, digit, and pattern type, were tabulated and compared between cases, controls and UFM. Overall, cases had lower ridge counts compared to both controls and UFM. Cases also had lower per digit mean TRC for all digits, with digits III and IV the most significantly different (p0.05). To evaluate individual pattern size differences, we also calculated the mean ARC of each fingerprint pattern type, regardless of digit location. A decrease in the size of ulnar loops (p=0.009) and a moderate increase in the size of whorls (p=0.067) was observed in cases. There is not clear evidence that the gradient of severity of the nail dysplasia is reflected in the dermatoglyphic phenotype. This is the first study of the quantitative dermatoglyphic phenotype in NPS. These results indicate that the mutations in the LMX1B gene and its affect on distal limb development result in quantitative changes of the dermatoglyphic phenotype of NPS. Supported by NIH grants R01-DE016148, P50-DE016215.

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Congenital Bilateral Perisylvian Syndrome (CBPS): familial recurrence, clinical and psycholinguistic aspects correlated with MRI. *I.L. Brandão-Almeida[1], M. M. Guerreiro[2], S. R. V. Hage[3], C. Guimarães[2], M. A. Montenegro[2], F. Cendes[2], I. Lopes-Cendes[1].* 1) Dept Medical Genetics, Campinas State University, Campinas, Brazil; 2) Dept Neurology, Campinas State University, Campinas, Brazil; 3) Dept Speech Pathology, University of São Paulo, Bauru, Brazil.

Congenital bilateral perisylvian syndrome (CBPS) is a malformation of cortical development most frequently caused by perisylvian polymicrogyria (PMG), with an increased number and smaller cortical folds than normal brain. We aimed to correlate the clinical and neuroradiologic data with the psycholinguistic aspects in a large group of patients with CBPS. Thirty two patients (twenty two males and 10 females) with mean age of 17.4 years (range, 5-65 years) were studied. Fourteen were isolated patients and 18, belonging to 5 unrelated kindreds, had familial recurrence of the disease. MRI scanning, neuropsychological tests and language evaluation was obtained in all patients. Their cognitive level was assessed using one of the Wechsler intelligence scales. Language testing was performed according to semi structured protocol that evaluated vocabulary, free conversation, and repetition. **Results:** Twenty-eight patients had polymicrogyric appearance of perisylvian cortex, characterizing the syndrome named PMG. One had unilateral PMG and only 3 patients had normal perisylvian region (one of whom had bilateral parietal posterior PMG and two had bilateral frontal PMG). Significant prenatal events, were reported in 14 patients. All patients had similar neurologic dysfunction, mainly primarily pseudobulbar paresis. Hemiparesis was present in only 3 patients. Seven of our patients had epilepsy. Specific language impairment was found in 13 patients and psychological assessment showed that global cognitive deficit was not present in most of them, although they usually have lower verbal IQ when compared to their performance IQ. None of our patients had facial dysmorphic features.**Conclusions:** Severity of clinical manifestations in CBPS is correlated with extent of cortical involvement. Epilepsy is not a common feature in patients with CBPS. Most of our patients have speech delay or language difficulties,including dyslexia.

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Maternal Plasma Folate and Cleft Lip and Palate in Philippines. *M. Tolar[1], A. Al-Jabeiti[2], T. Pawar[2], M. Tolarova[2].* 1) Pediatrics, PCRC Core Laboratory, Univ California, Children,s Hospital, San Francisco, CA; 2) Orthodontics, Craniofacial Genetics, University of the Pacific A. A. Dugoni School of Dentistry, San Francisco, CA.

The etiology of nonsyndromic orofacial clefts (OFCs) is multifactorial, involving genetic and environmental factors. A low folate intake and disturbances of the folate metabolic pathway have been shown to play a critical role in the etiology of dysraphic anomalies such as neural tube defects, orofacial clefts, and conotruncal heart defects. Our pilot study was focused on the comparison of plasma folate levels (PFLs) between two groups of women in Cebu City, Philippines - those who had a child affected with cleft lip and palate, and those who had a healthy child. Our sample consisted of 57 mothers of patients affected with OFC and 57 mothers of unaffected children. The Immulite 1000 analyzer (Diagnostic Products Corporation, CA) was used for quantitative measurements of PFLs in plasma. The normal range of folate concentration in human plasma is 3-17 g/L. The mean PFL for mothers of cases was 3.44 g/L (SD=1.6), while the mean PFL for control mothers was 4.04 g/L (SD=1.43). The difference was statistically highly significant (t-test; p= 0.0001). There was a tendency for higher frequencies of case mothers in the low PFL groups and for higher frequencies of control mothers in the high PFL groups. Chi square test for trend confirmed a dose dependent effect (2 = 5.1389; p=0.0234). The highest risk for having a child with a cleft was found for mothers with PFL lower than 2.68 g/L. A support of Rotaplast International, Inc., for sample collection and a support of UOP A. A. Dugoni School of Dentistry for acquisition and analysis of data are acknowledged.

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Ovarian dysfunction among premutation carriers of the *FMR1* gene. *S.L. Sherman, A.K. Sullivan, E.G. Allen, K. Harkreader, J. Brown, C. Small, M. Marcus.* Emory Univ, Atlanta, GA.

Premature ovarian failure (POF), defined as cessation of menses before the age of 40, occurs in 1% of women in the general population. Women who carry the *FMR1* premutation allele have a significantly increased risk for POF: about 13% have POF and the penetrance depends, in part, on the CGG repeat size. Our goal is to determine the reproductive profile of women who carry the premutation to identify clues about the cause of their ovarian dysfunction. For our current study sample of 466 non-carriers and 339 premutation carriers, we have obtained *FMR1* repeat size and transcript level, X-inactivation status, reproductive history using a structured interview and, in a subset of women, serum FSH levels. Traits consistent with a diminished oocyte reserve would include increased dizygotic twinning rate, increased spontaneous abortion, and decreased fertility. Among premutation carriers, we found a non-significant increase in dizygotic twinning and no evidence for increased miscarriages or clinically significant decreased fertility. We also examined menstrual cycle characteristics. There was no significant difference in age at menarche among groups. We found that carriers showed a significantly shorter cycle length ($p<0.001$), and, for those not on HRT, a significantly shorter bleed length compared to non-carrier women ($p=0.013$). However, premutation carriers did not have a greater variability in cycle length, a trait that is associated with infertility. We have also examined the effect of smoking, a known endocrine disruptor, on age at menopause. By survival analysis, we found a significant difference in the age at menopause for women who have ever smoked compared to women who have never smoked for both premutation carriers ($p=0.004$) and for non-carriers ($p<0.001$); the interaction between smoking status and premutation status was not significant. We hypothesize that these symptoms are due to increased follicular atresia as a result of the accumulating toxic effect of expanded *FMR1* transcripts.

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Novel mutation in the gamma-D crystallin gene (CRYGD) associated with polymorphic congenital cataract. *O.V. Plotnikova[1], A.P. Grigorenko[1], E.K. Ginter[3], E.I. Rogaev[1,2].* 1) Mental Health Research Center, RAMS, Moscow, Russian Federation; 2) Brudnick Neuropsychiatric Research Institute, UMASS Medical School, Worcester, MA,USA; 3) Research Center of Medical Genetics, Institute of Clinical Genetics, RAMS, Moscow, Russian Federation.

Aims: Previously we described a new form of cataract termed polymorphic congenital cataract (PCC) and mapped the locus for PCC to the gamma-crystallin gene (CRYG) cluster on human chromosome 2q33-35 in large unique large pedigree from genetic isolate of Turkmen origin in Middle Asia (Rogaev E.I. et al., Human Molecular Genetics, 1996). The cataract is characterized by irregular opacity with variable location between the fetal; nucleus of lens and the equator. The color of opacity also varies from shining crystal-like to snow-white. The genetic isolate and PCC pedigree is characterized by complex ethnic origin, high endogamy and a high coefficient of inbreeding (>3%). The frequency of the PCC gene in this population is 2.47%. We searched for the gene and mutation causing the PCC. Methods: In a survey of the population, at least 34 affected sibships with PCC were identified. Large pedigree with 105 individuals with PCC was reconstructed. 100 members from Turkmen pedigree were used for genotyping analysis. Coding regions of the CRYGA, CRYGB, CRYGC and CRYGD genes were amplified by PCR using genomic DNA from affected and non-affected individuals in this pedigree. The PCR products were sequenced and analyzed for heterozygous or homozygous variations. Results: Novel mutation was identified in gamma-D crystallin gene (CRYGD) that segregate with PCC in this pedigree. The mutation resulted in amino acid substitution of the encoded gamma-D crystallin protein. All affected individuals were found to be heterozygous for this missense- mutation confirming autosomal-dominant inheritance of the PCC. Three common single nucleotide polymorphisms in CRYGB and CRYGD were also detected. Conclusion: This is the first report of a mutation in the CRYGD protein resulting in autosomal dominant polymorphic congenital cataract, which is characterized by wide variations in phenotype of non-nuclear lens opacities.

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539 Cases of Gastroschisis: Increased risk of aneuploidy among fetuses of older mothers. *D. Durand[1], A. Cronister[2], R. Stoessel[3], D. Abad[1].* 1) Genzyme Genetics, Miami, FL; 2) Genzyme Genetics, Phoenix, AZ; 3) University Perinatal Associates, Palm Beach Gardens, FL.

Purpose: To determine the chromosome abnormality rate, gender differences and maternal age characteristics of amniocentesis samples submitted with the indication of gastroschisis. Methods: The Genzyme Genetics Laboratory database was queried from 2000 - 2005 to identify all amniotic fluid samples submitted with a referral indication of gastroschisis. Information on cytogenetic results, fetal gender and maternal age was documented and statistically analyzed. Results: 543 amniotic fluid samples with a referral indication of gastroschisis were identified. Cytogenetic analysis was not possible on 4. Of the remaining 539 the mean maternal age was 22.81 years among chromosomally normal fetuses and 33.08 among chromosomally abnormal fetuses. This difference was statistically significant ($p<0.0001$). Among the chromosomally normal group, there were 274 males and 255 females (a male to female ratio of 1.08:1). Ten chromosome abnormalities were identified, seven of which were male. Nine were trisomes and one was 45,X/46,XX karyotype. The overall aneuploidy rate was 1.86%. There was no statistically significant difference between the aneuploidy rates of male fetuses (2.49%) compared to female fetuses (1.16%) ($p=0.3437$). Discussion: Our data confirms the previously reported 1-2% aneuploidy rate associated with gastroschisis. The male to female ratio of 1.08:1 is also consistent with previous reports. Of interest, all but two of the trisomy cases were male and the mean maternal age in the aneuploidy cases was significantly elevated compared to maternal age among fetuses with normal karyotypes. Conclusions: We present data on 539 fetuses with gastroschisis. To our knowledge, this represents the largest series of prenatally diagnosed gastroschisis cases reported. This is also the first report of mosaic Turner syndrome associated with gastroschisis. Based on this data, it appears the risk of aneuploidy in fetuses with gastroschisis is increased in fetuses of older mothers.

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Extreme phenotypic variability and a new mutation in HLXB9 in a Currarino syndrome kindred. *R. Wang[1], J.R. Jones[2], S. Chen[3], R.C. Rogers[2], M.J. Friez[2], C.E. Schwartz[2], J.M. Graham[1].* 1) Dept. Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Greenwood Genetics Ctr, Greenwood, SC; 3) Dept. Pediatric Surgery, Cedars-Sinai Medical Ctr, Los Angeles, CA.

Currarino syndrome (MIM #176450) is an autosomal dominant disorder of hereditary sacral dysgenesis. Partial sacral agenesis, presacral mass, and anal malformations comprise the classic triad, but gastrointestinal, genitourinary, and neurologic anomalies are common. The syndrome is caused by mutations of the hox gene HLXB9 located on 7q36. It has three exons, encoding a 403 amino-acid protein with a homeodomain spanning residues 243 to 302. To date, 25 different mutations have been identified: all 9 missense mutations lie within the homeodomain; the rest are nonsense, frameshift, splice site mutations or whole-gene deletions. No genotype/phenotype correlations exist, and there is marked inter and intrafamilial variability. We present a family with a newly discovered mutation in HLXB9. The proband is a female without anal or sacral malformation presenting with perianal sepsis. Imaging revealed an inflammatory presacral mass, normal sacrum, spinal cord, and urogenital structures. Biopsy showed fibrous and neuronal elements. Her father has episodic constipation, but examination was unremarkable without malformation or hypertonia. Imaging showed normal sacrum without presacral mass or meningocele. His sister has bicornuate uterus, presacral teratoma, recurrent urinary tract infections, and perianal sepsis. Her daughter had neonatal bowel obstruction and anal stenosis requiring sequential dilatation, bicornuate uterus, and chronic constipation that has improved with age. Her daughter has the Currarino triad and required a diverting colostomy due to severe neonatal bowel obstruction. She recently developed recurrent urinary tract infections and hydronephrosis was found. Mutation analysis revealed that these patients had a previously unreported nonsense mutation, E283X, absent in tested asymptomatic first-degree relatives. This family provides additional information on the degree of intrafamilial variability associated with HLXB9 mutations, with phenotypes ranging from asymptomatic to severely affected.

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Redefining the Clinical Phenotype in Angelman Syndrome Using Microarray-Based Comparative Genomic Hybridization Testing in Children with Known Deletions. *S.U. Peters[1], T. Sahoo[2], A.L. Beaudet[2], J. German[2], L.M. Bird[3], R. Barbieri-Welge[3], T.J. Bichell[3], C.A. Bacino[2].* 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Dept Mol & Human Genetics, Baylor Col Medicine, Houston, TX; 3) Dept Pediatrics, San Diego Children's Hosp., San Diego, CA.

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe mental retardation, ataxia, and a happy disposition. Although there are four different molecular types of Angelman Syndrome, deletions of the 15q11-q13 region account for approximately 70% of the Angelman Syndrome patients. These deletions are usually detected by fluorescent in situ hybridization (FISH) studies. The deletions can also be sub-classified based on their size into Class I and Class II with the former class being the larger of the two. We have studied 30 children with Angelman Syndrome with known deletions using a microarray-based comparative genomic hybridization (CGH) assay and analyzed their phenotypic severity, especially expression of the autism phenotype, in order to establish clinical correlations. Based on the analyses of 22 children thus far, those with larger, Class I deletions have a more severe phenotype than children with smaller, Class II deletions. Specifically, children with Class I deletions were significantly more likely to meet criteria for autism ($p=.01$), and had lower cognitive scores ($p<.05$) as compared to children with Class II deletions. Trends were noted with Class I children receiving lower scores for expressive language ($p=.06$) and receptive language scores ($p=.07$). Although no differences in seizure severity were noted between the two groups, children with Class I deletions required more medications to control their seizures in comparison to the Class II group ($p = .046$). There are four known genes (NIPA 1, NIPA 2, CYFIP1, & GCP5) that are deleted in class I but not class II deletions, thus raising the possibility of a role for these genes in autism as well as the development of expressive language skills in particular. In light of these genotype/phenotype correlations, implications of our findings for re-defining the clinical and behavioral phenotype in Angelman syndrome will be discussed.

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A Structure-Function Study of MID1 Mutations Associated with a Mild Opitz Phenotype. *L. Mnayer[1], S. Khuri[1], H. Merheby[1], G. Meroni[2], L. Elsas[1].* 1) The Dr. John T. Macdonald Foundation Center for Medical Genetics, University of Miami, FL; 2) Telethon Institute, Naples, Italy.

The X-linked form of Opitz syndrome (OS) affects midline structures and produces a characteristic, but heterogeneous phenotype that includes mental retardation, hypertelorism, broad nasal bridge, widows peak, cleft lip/cleft palate, congenital heart disease, laryngotracheal defects, and hypospadias. The MID1 gene is implicated in OS, and maps to Xp22 encoding a 667 amino acid protein that contains a ring finger motif, two B-box zinc fingers, a coiled coil, a fibronectin type III (FNIII) domain, and a B30.2 domain. OS is seen as a severe disorder; however, here we describe a case of an intelligent male who has a milder phenotype. He presents with hypertelorism, broad nasal bridge and widows peak, hypospadias, pectus excavatum, and a surgically corrected tracheo-esophageal fistula, but without mental retardation, cleft lip/palate or heart disease. We identified a novel mutation (P441L) which we localized to the FNIII domain of MID1. Unlike almost all other reports of MID1 missense mutations associated with OS, P441L presents with a milder phenotype. While OS phenotypes have been attributed to mutations in the C-terminal part of MID1, little is currently known about the structure function relationships of MID1 mutations, and how they affect phenotype. A literature review suggests that missense mutations within the FNIII domain of MID1 are associated with a milder presentation of OS than missense mutations elsewhere in MID1. All truncating mutations (frameshift, indels) lead to severe OS. We used homology modeling of the MID1 FNIII domain to investigate possible structure-function changes by missense mutations. Preliminary results suggest that these mutations cause disruption of protein-protein interactions, either within MID1 or between MID1 and other proteins. We correlate the findings to the resulting milder phenotype and absence of CNS involvement, which is most likely due to differences in MID1 expression during embryonic development. It may be that the FNIII domain is important for midline differentiation after neural tube and palatal structures are completed.

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Musculoskeletal findings in the older patient with Cornelia de Lange Syndrome. *A.D. Kline[1], C.P. Pichard[2], I. Krantz[3], P.D. Sponseller[2].* 1) Harvey Inst Human Gen, Greater Baltimore Medical Ctr, Baltimore, MD; 2) Dept Orthopedics, Johns Hopkins Univ School Medicine, Baltimore, MD; 3) Div Human Gen, The Children's Hosp Philadelphia, Philadelphia, PA.

Individuals with Cornelia de Lange syndrome (CdLS) exhibit a variety of musculoskeletal anomalies, ranging in severity from minor hand findings to severe reduction defects with single digits. Although specific anomalies have been described, management and treatment guidelines have not been evaluated, nor have aging and limb involvement been studied or correlated with intelligence and severity. Through an ongoing clinical study to assess aging, orthopaedic involvement has been evaluated in 36 adolescents and older patients with CdLS to date, with 44% over 18 years of age. 33% have absent forearm(s); higher than previously noted. 77% were ambulatory without assistance. More severe findings were seen in individuals with a lower range of intelligence, similar to previous reports. Several anomalies not previously recorded were seen in our patients. Bunions were found in 72%, and leg length discrepancy in 31%; both could be a function of aging. Delayed skeletal maturity was noted in 10%. Orthopedic procedures were performed on 36% of the patients, more on the lower than upper extremity, and more procedures were done in children with lower birth weights and higher intelligence. Findings indicative of possible premature aging in CdLS include degenerative joint disease, osteopenia and fractures in the late teens or early 20's. Further investigation into etiology for these is ongoing. 33% of our patients have undergone mutation analysis of the NIPBL gene, and comparisons of detectable molecular changes with musculoskeletal findings and severity of involvement will be made.

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Gene polymorphisms or haplotypes in the Endothelial cell protein C receptor as potential risk factor for Idiopathic recurrent pregnancy loss: a population-based case-control study. *D. Bercovich[1,2], D. Levi[1], G. Sarig[3], C. Schochat[1], G. Tenenbaum[3], B. Brenner[3].* 1) Human Molec Genetics & Pharm, MIGAL-Galilee Technology Ctr, Kiryat-Shmona, Israel; 2) Tel Hai Academic College; 3) Hemostasis Unit, Rambam Medical Center, Haifa, Israel.

Idiopathic recurrent pregnancy loss (RPL) is associated with maternal thrombophilia but still 30-50% of RPL cannot be explained and might be associated with new or unknown thrombophilia. Moreover, the potential role of maternal thrombophilia in neonatal thrombosis is not established. The endothelial cell protein C receptor (EPCR) functions as an important regulator of the protein C anticoagulant pathway by binding protein C and enhancing activation by the thrombin-thrombomodulin complex. A soluble form of EPCR circulates in plasma and inhibits APC anticoagulant activity, thus, functions as procoagulant component. In order to find new thrombophilic risk factors for RPL, we studied the EPCR gene polymorphisms and soluble EPCR (sEPCR) levels in women with RPL compared to healthy controls. Analysis of three DNA alterations (SNP) in the EPCR gene; 1651 C>G in the 5' UTR, 6936 A>G Ser219Gly and 7014 C>G in exon 4, were evaluated in 215 women with RPL and 232 women without pregnancy complications. Differences in allelic frequencies were found between women with RPL and controls in SNP 1651 C/G (C 0.36, G 0.64 and C 0.23, G 0.77, respectively, OR=0.54, P=0.0016) and SNP 7014 C/G (C 0.23, G 0.77 and C 0.33, G 0.67, respectively, OR=1.5, P=0.0086). haplotype C;A;G (SNPs: 1653, 6936 & 7014 respectively) were significant more frequent in the RPL group (OR=1.83, P=0.023) and haplotype G;A;C were significant more frequent in the control group (OR=0.49, P=0.028). Genotype 6936AG was found to be associated with high levels of sEPCR; mean levels of sEPCR in women with genotype 6936AA was 120Â37 ng/ml compared to 250Â73 ng/ml in genotype 6936AG, P=0.001. Genotype 6936AG frequency (14%) as well as sEPCR levels did not differ between women with RPL and controls (153Â70 ng/ml and 135Â61 ng/ml, respectively). In conclusion: these results suggest that SNPs 1651 C/G and 7014 C/G in the EPCR gene might be associated with RPL.

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Molecular genetics of Warburg Micro Syndrome (microphthalmia, cataract, microcephaly and microgenitalia).

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Warburg Micro syndrome is a severe autosomal recessive disorder characterised by developmental abnormalities of the eye and central nervous system and microgenitalia. We previously undertook a 10cM genome -wide linkage scan in eight consanguineous families to map the first Micro locus (WARBM1) to 2q21.3. Homozygous inactivating mutations in RAB3GTPase activating protein (RAB3GAP) were then found in 12 of 18 Micro syndrome families. In mouse we found significant expression of Rab3GAP in the developing eye and brain. Mutation analysis has now been performed in a further 10 families. This has not only confirmed that Micro syndrome is a heterogenous disorder but extended the phenotype associated with dysregulation of the Rab3 pathway. Further clinical investigations to delineate the endocrine and neuroradiological features of the condition have been undertaken. RAB3GAP has a critical role in regulating the function of the four Rab3 family members that are implicated in neurotransmitter and hormone release by calcium mediated exocytosis. The Micro syndrome phenotype demonstates that RAB3GAP is essential for the normal development of the brain , eye and genitalia. We hypothesise that the underlying pathogenesis of Micro syndrome is a failure of exocytic release of ocular and neurodevelopmental trophic factors.

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Detection and mapping of chromosome aberrations in congenital diaphragmatic hernia patients using array CGH. *A. Slavotinek[1], A. Moshtefi[1], R. Davis[2], E. Leeth[3], R. Keller[1], K. Nobuhara[4].* 1) Dept Pediatrics, U585P, Univ California, San Francisco, San Francisco, CA; 2) Comprehensive Cancer Center, UCSF, CA; 3) Evanston Northwestern Healthcare, Evanston, IL; 4) Dept Pediatric Surgery, UCSF, CA.

Congenital diaphragmatic hernia (CDH) is a common birth defect with a high mortality and morbidity. We are currently performing array CGH on DNA from probands with syndromic and non-syndromic CDH to detect and map chromosome aberrations. We have used the HumArray 3.1 BAC array with an average resolution of 1.4 Mb to study 30 patients, 10 with syndromic CDH, 15 with non-syndromic CDH and 5 with CDH and previously known cytogenetic aberrations. In four of these patients, we have mapped 15q26.2 deletions that overlap with the critical 5 Mb region for CDH delineated by Klaassens et al. (2005). These patients all have features consistent with 22 reported patients who had 15q24-15qter deletions, allowing characterization of a recognizable gene deletion syndrome comprising CDH, intrauterine growth retardation, pulmonary hypoplasia, cardiac defects, a single umbilical artery and talipes/rockerbottom feet. In 2 patients with known cytogenetic deletions, we have mapped an 8 Mb deletion of 4p16 and a 4-10 Mb deletion of 1q41-1q42.2. Monosomy for these chromosome regions has previously been reported with CDH, but the critical regions had not been molecularly defined. We conclude that array CGH is vital to exclude submicroscopic chromosome rearrangements in patients with CDH and additional anomalies. Klaassens et al., AJHG 2005;76epub.

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Genetic and life-course determinants of bacterial infections: a birth cohort study over 30 years. *M.-R. Järvelin[1], P. Elliott[1], M. Levin[3], A.-L. Hartikainen[4], U. Sovio[1], J. Key[1], S. Cheng[5], J.A. Summerfield[2].* 1) Department of Epidemiology and Public Health, Imperial College, London, UK; 2) Division of Medicine, Imperial College, London, UK; 3) Division of Pediatrics, Obstetrics and Gynaecology, Imperial College, London, UK; 4) Department of Public Health and General Practice, Oulu, Finland; 5) Department of Human Genetics, Roche Molecular Systems, Alameda, CA, USA.

Objective. To test the hypothesis that polymorphisms in the MBL and TNF genes contribute to the risk of bacterial infections. **Methods.** A nested case control study in The Northern Finland Birth Cohort of 12,058 live births followed-up to age 31. Data on life-course and admissions to hospital with severe/recurrent bacterial infection and with non-infectious diseases (classified by ICD 8-10) based on records from neonatal period, questionnaires at ages 1, 14 and 31 years and national cause-of-death and hospital discharge registers up to age 31. 327 cases (157 severe) were matched with 651 controls by sex, maternal education and parity. **Results.** MBL haplotypes were not associated with infection risk. The rare TNF (-308 A/A) genotype associated with a two to three fold risk of bacterial infection compared to G/G genotype; unadjusted OR 2.9 (95% CI 1.04-8.3), 3.01 (1.1-8.5) adjusted for birth weight and 2.2 (0.7-6.5) further adjusted for early life factors. Many non-infectious diseases (respiratory diseases, congenital malformations) were associated independently with risk of infection, and adjustment for these diseases generally attenuated the genotype-phenotype association. TNF (-308 A/A) genotype was associated with higher risk for blood and skin diseases. Low birth weight and low maternal body mass index were independently associated with risk of bacterial infection (lowest decile vs. the rest, OR 2.1 (1.3-3.6) and OR 1.7 (1.08-2.76), respectively). Farmer background reduced the risk of infections (farmers vs. highest 3 classes, OR 0.6 (0.4-0.9)). **Conclusions.** Lifecourse exposures and genetic polymorphisms are important determinants of bacterial infections. This study illustrates the complexity of genetic analyses of multifactorial disorders.

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Autosomal dominant Peters anomaly in a large family without causative mutations in PAX6 and CYP1B1 genes.

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Peters anomaly (MIM 604229) includes central corneal opacity and iridocorneal adhesions due to defects in the posterior corneal stroma, Descemets membrane and corneal endothelium. It occurs as an isolated abnormality or in association with various ocular defects and congenital anomalies. Familial type is usually autosomal recessive however, dominant inheritance is rarely reported. A few cases have been associated with mutations in PAX6, RIEG, PITX3 and CYP1B1. Here we report the phenotypic presentation in a multiplex family that fits in the Peters anomaly spectrum. The complete pedigree consists of a total of 44 members(16 affected)in an autosomal dominant inheritance pattern. The six examined affected members demonstrate bilateral microcornea, corneal opacity, iridocorneal adhesions, nystagmus and strabismus without glaucoma or cataract. PAX6 gene mutations were previously excluded based on sequencing analysis of the coding region of the gene. In this study, we analyzed the coding region (exon 2 and 3) of CYP1B1, the major known cause of primary congenital glaucoma. No causative mutation except two reported SNP changes (V432L and a silent chance D449D) has been identified. Compound heterozygosity in codon 432 (V432L/R) is suggested to be associated Peters anomaly (Molecular Vision 2005; 11: 66-70). Published data on CYP1B1 polymorphisms show that the frequency of leucine allele in healthy Turkish control population is 71%, therefore the observed heterozygous V432L change in this study is not consistent with a causative relationship. In conclusion, the lack of cataract and glaucoma in Peters anomaly, with presence of microcornea, nystagmus and strabismus suggests a clinical phenotype in between autosomal dominantly inherited cataract-microcornea syndrome (MIM 116150) and Peters anomaly itself which has variable patterns of inheritance. PAX6 and CYP1B1 genes are less likely to be responsible for this phenotype.

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MORM syndrome (mental retardation, truncal obesity, retinal dystrophy, and micropenis), a new autosomal recessive disorder, links to 9q34. *D.J. Hampshire[1], K. Springell[1], M. Ayub[2], E. Roberts[1], H. Jafri[3], Y. Rashid[3], J. Bond[1], J.H. Riley[4], C.G. Woods[5].* 1) Molecular Medicine Unit, University of Leeds, St James's University Hospital, Leeds LS9 7TF, UK; 2) Department of Psychiatry of Learning Disabilities, St Luke's Hospital, Middlesbrough TS4 3AF, UK; 3) Department of Obstetrics and Gynaecology, Lady Wellington Hospital, Lahore, Pakistan; 4) Discovery and Pipeline Genetics, GSK, New Frontiers Science Park North, Harlow CM19 3AW, UK; 5) Department of Medical Genetics, CIMR, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2BP, UK.

A consanguineous pedigree is described where fourteen individuals are affected with a novel autosomal recessive disorder which causes static moderate mental retardation, truncal obesity, a congenital non-progressive retinal dystrophy and micropenis in males. We have named this condition MORM syndrome. This disorder shows similarities to Bardet-Biedl syndrome and Cohen syndrome; however linkage to the known Bardet-Biedl (BBS1-8) and Cohen syndrome loci was excluded. Autozygosity mapping identified a single homozygous region shared by all affecteds on chromosome 9q34.3, with a maximum LOD score of 5.64. We believe this to be the first example of the identification of a sub-telomeric recessive locus by autozygosity mapping.

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The Pompe Registry: demographics, methods of diagnosis and preliminary genotype-phenotype correlation. *K. Sims.* Molecular Neurogenetics, Massachusetts General Hosp, Boston, MA.

Pompe disease is a rare, lysosomal disorder secondary to deficiency of alpha-glucosidase and glycogen storage in skeletal and cardiac muscle with variable disease progression. The Infantile-onset form presents in first months of life with hypotonia, generalized muscle weakness and cardiomyopathy followed by death from cardiorespiratory failure often by 1 year. A Late-onset form shows little cardiac involvement, and slower progression of muscle weakness. Eventual loss of ambulation and respiratory insufficiency causes early mortality. To better understand the natural course of this disease, a global Pompe Registry has been initiated.

Results: *Demographics:* Currently, 70 patients are enrolled in the Registry. 31 (44%) female, 39 (56%) male, 50 (71%) Caucasian, 2 (3%) Black, 2 (3%) Asian. Average age is 30.1 years with 40 (57%) patients > 20 years, 10 (14%)10-20 years, 20 (29%) 1-10 years and none < 1 year. 13 (19%) patients were diagnosed as Infantile-onset, mean age 7.4 months (0.9-18.4 months). 48 (69%) were diagnosed as Late-onset, mean age 31.8 years (1.3-64.0 years). *Diagnostic Data:*34 (49%)were diagnosed by DNA analysis and 48 (69%) by enzyme assay [29% lymphocyte, 26% fibroblast,15% leukocyte]. 44% of patients were tested using more than one diagnostic method. Muscle biopsies were performed on 34%. *Genotype Data:* Mutation analysis in 30 patients identified 11 unique mutations. Two occurred more than once: IVS1-13T>G (19/30, 63%) and IVS1-13T>C (2/30, 7%). Those with IVS1-13T>G were primarily Late-onset [84%] mean age 44.0 years (9.6-72.2 years).

Conclusions: Mutiple methods were used for diagnosis. DNA testing identified mutations in 20/30 [67%]. Data suggests a milder, Late-onset phenotype associated with common IVS1-13T>G mutation. Future Registry analysis may identify other correlates to clinical disease severity.

The Pompe Registry is a physician managed, Genzyme sponsored tool, designed to collect longitudinal patient data to improve understanding of this rare disease and enhance patient care..

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An epidemiologic evaluation of patients with multiple vertebral segmentation defects. *S.J. Drescher[1], M.H. Reed[1,2,3], A.E. Chudley[1,3], J.A. Evans[1,3].* 1) Department of Biochemistry and Medical Genetics; 2) Department of Radiology; 3) Department of Pediatrics and Child Health; Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

Introduction: Skeletal dysplasias involving segmentation defects of the spine and ribs have been described under many names including spondylocostal or spondylothoracic dysostosis, costovertebral dysplasia and Jarcho-Levin syndrome. These multiple vertebral segmentation defects (MVSDs) are clinically and radiographically heterogeneous, making genetic counselling for prognosis and recurrence risk difficult.

Methods: One aim of our study is to perform an epidemiologic evaluation of local cases with MVSDs, which will be useful for determining the nature and severity of these malformations. Using a scoring system for clinical and radiological information, we intend to perform cluster analyses to define groups of individuals with similar features. Our study population is being ascertained through review of literature and local cases obtained from the Genetics and Metabolism Program at our centre.

Results: Preliminary review of 84 local cases shows a 3:4 ratio of affected males to females. Most cases were livebirths, although several were detected prenatally and the pregnancies terminated. The finding of a number of cases with maternal diabetes will be followed as an etiologic factor. Although the association between maternal diabetes and caudal regression syndrome is well recognized, upper vertebral anomalies have been less well delineated in this group. Radiographically, defects were found at all levels of the spine, with thoracic anomalies being the most common. Cardiac, renal and limb malformations were those most commonly seen in assocation with MVSDs. Survival of liveborn cases varied from 15 minutes to more than 30 years.

Conclusions: These preliminary findings illustrate the heterogeneity of this population and the potential for identifying groups of individuals with similar features.

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Novel mutations in the TWIST Box anti-osteogenic domain of *TWIST1* associated with single-suture craniosynostosis. *M.L. Seto[1], J. Chang[1], M. Hu[1], A.V. Hing[1], K. Kapp-Simon[2], P.K. Patel[2], M.L. Cunningham[1].* 1) Pediatrics, Division of Genetics and Developmental Medicine, University of Washington, Seattle, WA; 2) Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL.

Craniosynostosis, the premature fusion of one or more cranial sutures, is a significant developmental disorder affecting 1 in 2500 live births. Single-suture fusion is the most prevalent form of craniosynostosis, with sagittal synostosis occurring in 1/5000 live births. We are conducting a multi-site, matched control, five-year longitudinal study of the neurobehavioral correlates of 250 infants with single-suture craniosynostosis. One focus of this study is screening each affected infant for mutations in candidate regions of genes known to cause craniosynostosis: *TWIST1*, *FGFR1*, *FGFR2*, and *FGFR3*.

Of 159 affected infants screened to date, we have found two mutations within the recently characterized TWIST Box, a functional domain of *TWIST1*. In humans, no TWIST Box mutations have been cited prior to this report. Our first patient has a nt.G556C mutation (which is expected to cause an Ala186Thr change) and unicoronal synostosis, facial asymmetry, and mild aortic valvular stenosis. Our second patient has a nt.C563T mutation (which is expected to cause a Ser188Leu change) and isolated (non-syndromic) sagittal synostosis.

While Saethre-Chotzen Syndrome is associated with *TWIST1* mutations, the association of isolated sagittal or non-syndromic craniosynostosis with a *TWIST1* mutation is a novel finding in humans. The Charlie Chaplin mouse, with a missense mutation in the same conserved residue as our second patient, also displays sagittal synostosis. The TWIST Box has recently been shown to interact with and directly inhibit *RUNX2* - a gene necessary and sufficient for osteoblast differentiation. Thus, we believe the TWIST Box mutations in our patients are causative of their single-suture craniosynostosis. Future studies will further elucidate whether our cases truly represent a genotype-phenotype correlation between TWIST Box mutations and non-syndromic, single-suture craniosynostosis.

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Analysis of *SLC35A3* in Patients with Congenital Vertebral Malformations. *N. Ghebranious[1], L. Ivacic[1], E. McPherson[1], FS. Jacobsen[1], T. Faciszewski[1], JK. Burmester[2], RD. Blank[3], CL. Raggio[4], I. Glurich[2], K. Meddaugh[1], V. Kumar[1], PF. Giampietro[1].* 1) Marshfield Clinic, Marshfield, WI; 2) Marshfield Clinic Research Foundation, Marshfield, WI; 3) University of Wisconsin and GRECC Service, William S. Middleton Veterans Hospital, Madison, WI; 4) Hospital for Special Surgery, New York, NY.

Due to the sporadic nature of congenital vertebral malformations, traditional linkage methods to identify genes associated with the disease are not possible and therefore, a candidate gene approach is more appropriate. More recently, cattle with a specific point mutation in the Solute carrier family 35 (UDP-N-acetylglucosamine transporter), *SLC35A3* gene have recently been shown to have complex vertebral malformation syndrome (CVM). This autosomal recessive condition is lethal in cattle and is associated with congenital vertebral malformations and a variety of other birth defects that parallel various human congenital malformation syndromes including VACTERL, Klippel-Feil and Goldenhar syndromes. Because of the close phylogenetic relationship between humans and cattle, we hypothesized that mutations in *SLC35A3* may be associated with vertebral malformations in humans. To determine whether *SLC35A3* plays a role in human vertebral malformations, we sequenced the entire coding region and splice junctions of the *SLC35A3* gene in a cohort of 27 patients with a wide array of vertebral malformations from the Marshfield Clinic and Hospital for Special Surgery patient population. No significant mutations were found in the *SLC35A3* gene in any of the patients in the cohort. Several possibilities may account for the absence of mutations in these patients: a) the *SLC35A3* gene is not associated with human vertebral malformation as it is in bovine; b) the gene may be redundant in humans and mutations in other gene family members may be associated with congenital vertebral maformations; c) the disease is highly heterogeneous with multiple genes associated with the disease and thus a larger sample size is needed; and/or d) albeit less likely, but mutations could be clustered in the non-coding region of the gene that was not covered in this analysis.

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Meier-Gorlin syndrome (MGS): Report of two cases. *T. Ben-Omran[1,3], F. Al-Murshedi[1], R. Babul-Hirji[1], D. Wherrett[2], D. Chitayat[1,3].* 1) Clinical/Metabolic Genetic, Hosp Sick Children, Toronto, ON, CanadaÁâ; 2) Division of Endocrinology, Department of Pediatrics, The Hospital for Sick Children,; 3) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

The Meier-Gorlin syndrome is a rare autosomal recessive disorder, characterized by severe growth retardation, bilateral microtia, micrognathia, and aplasia or hypoplasia of the patellae. Additional clinical findings include early closure of cranial sutures, microcephaly, low-set and simple ears; small mouth, full lips, cleft or highly arched palate; genitourinary tract anomalies, and various skeletal anomalies. Approximately 23 cases have been reported thus far. We report two unrelated patients with Meier-Gorlin syndrome. Patient 1: An 18-year-old boy was born at term to healthy, nonconsanguineous Caucasian parents. Antenatal ultrasound at seven months gestation revealed intrauterine growth retardation. Birth weight was 1999g (<3%) and length 44 cm (<3%). He was noted to have ambiguous genitalia and dysmorphic features, including bilateral microtia, epicanthic folds, hypertelorism, broad nasal bridge, and micrognathia and developed sensorineural hearing loss due to Mondini malformation, and has mild optic nerve hypoplasia. The karyotype was 46,XY. A skeletal survey, at 14 years of age, showed rudimentary right patella, abnormal fusion of the lunate and pisiform bilaterally, and mild cervical and thoracic scoliosis. Patient 2: This female patient is the first child born at term to healthy, non-consanguineous parents of African/Spanish ancestry. Antenatal ultrasound at five months gestation revealed IUGR. Birth weight was 2kg, length 43cm and OFC 32.5cm (all <3%). She had bilateral microtia, hypertelorism, and VSD and PDA on echocardiography with mild mitral incompetence. Skeletal survey showed absent patella. MGS is an AR condition with variability of ethnic origin. Our patients add to the panethnicity of this condition and expand the clinical manifestations to include ambiguous genitalia and Mondini malformation. Further studies are being done to delineate the gene associated with this condition.

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Spectrum of Mitochondrial DNA Mutations in 18 Russian/Siberian Families with Lebers Hereditary Optic Neuropathy (LHON). *R.I. Sukernik[1], N.V. Volodko[1], M.A. Lvova[1], E.B. Starikovskaya[1], O.A. Derbeneva[1, 2], V. Procaccio[2], M.D. Brown[3], D.C. Wallace[2].* 1) Institute of Cytology and Genetics, SBRAS, 10, Lavrent'ev Ave, Novosibirsk, Russian Federation, 630090; 2) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, 2014 Hewitt Hall, Irvine, CA 92697-3940; 3) Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.

Subacute onset in young adults, bilateral, optic atrophy is the primary clinical sign of LHON, which is caused by missence mutations in the mtDNA genes encoding complex I subunits of the respiratory chain. We summarize results of the molecular genetic study of 18 genealogically unrelated families manifesting maternally transmitted visual failure consistent with LHON. The procedure entailed standard RFLP tests and mtDNA complete sequence analysis. Of 18 LHON families, 14 (78%) harbored common LHON mutations (G11778A, G3460A, and T14484C). The mutation G11778A identified in 8 of 9 Caucasian families, was preferentially associated with haplogroup TJ, and more specifically J1, J2 or T2. On the contrary, the mutation G3460A was confined to 3 indigenous families, Tuvan, Evenki, and Altaian, with their mtDNA types belonging to east-Eurasian lineages C3, D5 and D8, respectively. Heteroplasmy was detected in patients and their maternal relatives in two families, and in one of those (large Tuvan family) we were able to trace the origin of a de novo mutation. The mutation T14484C revealed in two extended pedigrees was associated with either haplogroup J1 or M9. In addition to these three primary mutations, a number of unique mutations (G3635A, C4640C, T10663C and A14619G) has been identified. The mutation A14619G (ND6/F19L) is a novel candidate mutation that has not been found in haplotype-matched or non-haplogroup (U4) control mtDNAs. The G14279A (ND6/S132L) is another novel mtDNA mutation revealed within the same geographic area (Zhadanov et al. 2005). Overall, it implies that this part of Siberia is conspicuous for variety of rare deleterious mtDNA mutations (G3635A, C4640C, G14279A, and A14619G) contributing to LHON expression.

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Re-evaluation of Individuals with FXS After 10 Years (1995-2004) of Molecular Diagnostic Experience in a Tertiary Turkish Center. *E. Tuncbilek[1], Y. Alanay[1], D. Aktas[1], K. Boduroglu[1], E. Utine[1], B. Volkan-Salancı[1], M. Alikasifoglu[1], Hacettepe FXS Study Group[2].* 1) Clinical Genetics, Hacettepe University , Ankara, Turkey; 2) Faculty of Medicine, Hacettepe University, Ankara, Turkey.

Fragile X syndrome (FXS) is the most common inherited cause of mental retardation. Molecular diagnosis is available since 1991. At Hacettepe, 1600 DNA samples were tested and 107 families (126 FM individuals) have been identified in the past 10 years . Among these 106 were male probands with mental retardation. Only one family was identified due to premature ovarian failure (POF). Hacettepe University FXS Study Group was established in 2004. This study was constructed to overview the phenotypic features of our whole group reviewing their medical records. The reviewed records enabled us to see the evolution of age at diagnosis. The mean age at diagnosis has decreased from 8.08 yrs between 1995 and 1999, to 6.92 yrs between 2000 and 2004, and to 5.7 yrs in 2004. Prenatal diagnosis was available for 15 families (14%). Following this review 24/106 families were recruited for follow-up visits by the FXS Study Group. The physical and behavioral features were re-evaluated. Family history suggesting POF(58.7%) and FXTAS(4.5%) were questioned. Mean age at diagnosis was 5.73. Mean IQ was 49.720.4(range 25-90). Mean diagnostic score was 15.73. EEG abnormality was detected in 37.5% without previous seizure. Pervasive disorders were present in 31.8%, while oppositional defiant disorder and ADHD were diagnosed in 27.3%, and 22.7%, respectively. Nearly 50% of the patients had a cardiac abnormality, most common being MVP or mitral insufficiency (13.6%). Routine ENT examination revealed opaque ear drum in 23.8% with minimal conductive hearing loss. All were seen by a speech therapist. Strabismus was present in 1/23 patients. The results of this study are in correlation with previous reports on clinical problems in FXS. We emphasize the importance of multidisciplinary approach for the clinical problems in FXS. The high rate of family history for POF supports data suggesting routine screening for FMR1 premutation in idiopathic POF and indicates that consciousness should be increased among gynecologists.

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Testing for subtelomeric abnormalities reveals a candidate region for Rubinstein Taybi syndrome. *E. Baldwin[1], J.A. Martinez[2], E. Crombez[2], S. Brodie[2], V. Vandergon[3], M. Fox[2].* 1) Children's Hospital Los Angeles, Los Angeles, CA; 2) University of California Los Angeles, Los Angeles, CA; 3) California State University Northridge, Northridge, CA.

A retrospective chart review at the University of California, Los Angeles (UCLA) identified the features and clinical presentations of individuals identified as carriers of a subtelomeric abnormality. Medical records for patients seen in the UCLA Genetics Clinic from January 2003 through December 2004 were reviewed. Of the 178 patients meeting criteria, 62 were tested by the UCLA Cytogenetics Laboratory for subtelomeric abnormalities using fluorescence *in situ* hybridization. Abnormalities were identified in seven (11.3%) of these individuals and included abnormalities of the 1p, 5q, 6q, 10q, 15q, and Xp subtelomeric regions. The patient identified with a cryptic 15q subtelomeric deletion had been previously given a clinical diagnosis of Rubinstein Taybi Syndrome (RTS) and lacked an identifiable mutation in the *CREBBP* gene. The patients phenotype included multiple cardiac anomalies, growth deficiency with a weight and height less than the third percentile, broad fingers and toes, and malar hypoplasia, consistent with a diagnosis of RTS. Candidate genes within the 15q subtelomeric region that could contribute to the phenotype in this patient include *NR2F2* and *MEF2A*. Both genes are involved in cardiac development, and *MEF2A* has been shown to interact with the gene for RTS. Considering that approximately 35% of individuals with clinical diagnoses of RTS will have a mutation in the *CREBBP* gene, the authors propose the subtelomeric region of 15q as a candidate region for a possible second gene contributing the RTS phenotype.

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Screening for subtelomeric aberrations in children with mental retardation from Navarra. *M. Artigas-López, A. Alonso, A. Pérez-Juana, A. Bengoa, A. Valiente, M.A. Ramos.* Genetics, Hospital Virgen del Camino, Pamplona, Navarra, Spain.

Subtelomeric chromosome rearrangements are an important cause of idiopathic mental retardation (MR). The study was based on patients from Navarra, included in the Regional Registry of Birth Defects (RACEHNA). From the preliminary results in 60 children with unexplained MR and a normal G-banded karyotype, using a set of 41 subtelomeric fluorescent DNA probes, 6 subtelomeric aberrations (10%) were found. Except for one family all parents tested negative. Case 1: 46,XX,del(1)(pter). A 18-year-old dysmorphic female with patent ductus arteriosus, growth failure, microcephaly, severe MR. Case 2: 46,XX,del(17)(pter). A 5-year-old girl with moderate MR and mild facial dysmorphic features. Terminal deletion 17p has a distinct phenotype with lissencephaly as the main feature, however few cases with MR and mild features and phenotypically normal subjects have also been reported. Case 3: 46,XY,del(10)(pter). A 5-year-old boy with short stature, bicuspid aortic valve, pyeloureteral duplicity, pectus excavatum, moderate MR and very mild dysmorphism. Parents were not available. However, his younger sister with mild delay and similar facial features had the same FISH result, suggesting the inheritance of the aberration. Case 4: 46,XY,dup(1)(qter)/del(2)(qter). A 14-year-old youngster, with hypotonia and severe micrognathia at birth, at present moderate MR, with a marfanoid body habitus. Case 5: 46, XY, del(2)(qter). A 3-year-old boy with esophageal atresia and tetrallogy of Fallot, MR, trigonocephaly, small deep set eyes and dysplastic ears. Case 6: 46,XY,del(1)(qter). A 4-year-old boy with ocular coloboma, hypogenitalism, short stature, oligogyric microcephaly, severe MR and dysmorphia. As reported in previous studies, our preliminary results indicate that subtelomeric aberrations are a leading cause of MR. Given the wide variation observed in the positive cases and the diversity of the chromosomal rearrangements identified, more studies are needed in order to characterize the distinct phenotypes and be able to diagnose them with the specific probe for the chromosome involved.

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Disruption of the *IL1RAPL1* gene associated with a pericentromeric inversion of the X chromosome causes MR and developmental delay. *S.S. Bhat[1], S. Ladd[1], F. Grass[2], B.R. DuPont[1], C.E. Schwartz[1], R.E. Stevenson[1], A.K. Srivastava[1].* 1) Greenwood Genetic Center, Greenwood, SC; 2) Department of Pediatrics, Carolina Medical Center, Charlotte, NC.

Characterization of X chromosomal rearrangements has proved to be a powerful tool in the identification of the X-linked mental retardation (XLMR) genes. We identified a 3 ¼-year-old male with MR and significant developmental delay. He also has a history of hand flapping and poor communication. Cytogenetic analysis revealed a pericentromeric inversion of the X chromosome: 46, Y, inv(X)(p22.1q13). The mother, who also has a history of learning difficulties and was in special class as a child, carried the same inversion on one X chromosome. Fluorescent in situ hybridization (FISH), using a panel of genomic clones, allowed the identification of a BAC clone from the Xp22.1 region spanning both the Xp22.1 and Xq13 breakpoints. The Xq13 breakpoint was narrowed to approximately 50 kb telomeric to the 3 end of *ACRC* gene. RT-PCR analyses detected no loss of expression of genes, *RPS4X*, *OGT*, *ACRC*, *CXCR3* and *KIAA2001*, present in the Xq13 breakpoint interval. Further analysis showed that the *IL1RAPL1* gene was disrupted by the Xp22.1 breakpoint and therefore its inactivation may be related to the mental retardation observed in the male patient. Defects of the *IL1RAPL1* gene have previously been shown to cause nonsyndromic XLMR. X-inactivation studies showed a random pattern of X-inactivation in the mother. It is likely that the learning disability in the patients mother is due to a partial loss of expression of the *IL1RAPL1* gene, which is disrupted by the inversion.

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A new method for *ATRX* gene mutation analysis using mismatch-specific endonuclease (Surveyor^{TM} nuclease). *T. Wada[1], Y. Fukushima[1], S. Saitoh[2].* 1) Dept. Medical Genetics, Shinshu Univ Sch Medicine, Matsumoto, Japan; 2) Dept. Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan.

X-linked -thalassemia/ mental retardation syndrome (ATR-X, OMIM#301040) is a syndromic form of XLMR (S-XLMR), and characterized by severe mental retardation in males, dysmorphic facies, hemoglobin H inclusions, genital abnormalities, skeletal abnormalities and characteristic posture and/or behavior. ATR-X is caused by a mutation in the *ATRX* gene, which is also involved in other S-XLMRs and also in NS-XLMR in males. Approximately 10% of named X-linked MR syndromes are candidates for allelism based on phenotypic findings. Recently we have suggested that *ATRX* gene mutations can cause MR in females, unless the mutated allele is properly inactivated.
To analyze the full range of disease-causing mutations for genetic counseling, and to establish the phenotype-genotype correlations, we established a new *ATRX* gene mutaton screening method using mismatch-specific endonuclease (Surveyor^{TM}). Genomic DNA samples from 12 patients with ATR-X from 12 families were used for experiments. We designed PCR primers for all 36 exons and flanking introns. The length of PCR products ranged from 300 to 600 base-pairs. The PCR products from a patient and a control were mixed, and denatured and hybridized to form hetero- and homoduplexes. The mixture was digested by mismatch-specific endonuclease (Surveyor^{TM}). The digested products were analyzed by a DHPLC (WAVE system).
We confirmed that all 11 known mutations of our patients were detected by this method, some of which had been difficult to be detected by the DHPLC method only. And we found other two novel mutations outside the ADD and the helicase domains, where most mutations of the ATR-X patients resided. This system does not need to set temperature conditions depending on mutation's site, and could be a superior method to the DHPLC method. We have started screening *ATRX* mutations for MR patients with or without ATR-X phenotype.

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A new cohort of MECP2 mutation screening in unexplained mental retardation: the occurrence of regression could be an indicator for routine molecular diagnosis. *A. Donzel, C. Thauvin-Robinet, V. Cusin, F. Mugneret, F. Huet, JR. Teyssier, L. Faivre.* Departement de Genetique, CHU de Dijon, France.

Besides to typical or atypical Rett, mutations in the MECP2 gene have been occasionally associated with severe encephalopathy, Angelman-like features, Prader-Willi-like features or unexplained X-linked mental retardation (MR). A few retrospective studies were designed to answer the question of whether or not an MECP2 mutation screening is indicated in boys with unexplained MR, and their compiled results showed a low frequency (1%) of MECP2 mutation in such cohorts. Here we performed MECP2 mutation screening by direct sequencing in a cohort of 146 well-characterized cases with MR (46 females, 100 males). The cohort included 2 groups depending if MR was isolated (92/146, group 1) or associated with other neurological features (54/146, group 2), including seizures, ataxia, microcephaly, autistic features and strabismus. None of the patients had stereotypes, regression nor irregular breathing. All patients had normal karyotype, Fragile-X study, MRI, plasmatic amino-acid and urinary organic-acid chromatographies. Two known pathogenic MECP2 mutations were found in two girls from group 2 (2/54, 4%), but none were found in group 1 with isolated non-specific MR (0/146, 0%). The R255X was found in a 3-year-old girl with psychomotor retardation and strabismus only, and the 1152-1192del41 mutation was found in another 2-year-old girl with psychomotor retardation, strabismus and ataxia at time of inclusion. Two years follow-up of both girls revealed some emerging features in favor of Rett-like phenotype, including loss of hand use, irregular breathing and manual stereotypes in case 1 and loss of speech in case 2. None of them had microcephaly or seizures. Ten non pathogenic sequence variants were also identified. We conclude that MECP2 mutations do not represent a major cause of non-specific MR and that MECP2 mutation screening could be useful in earlier diagnosis and genetic counseling in girls with ataxia or partial regression of motor or language skills with negative etiological investigations, even in the absence of progressive microcephaly and seizures.

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A study on the distribution of Hepatitis B virus genotypes in local isolates of HBV in Malaysia using PCR-RFLP analysis. *J.B. Chook[1], S.F Yap[1], J.A. Tan[2].* 1) Dept Pathology, University Malaya, Malaysia; 2) Dept Molecular Medicine, University Malaya.

More than 350 million individuals worldwide are persistently infected with Hepatitis B virus (HBV) and more than two billion people have serological evidence of past infection. Individuals chronically infected with HBV can either be asymptomatic or demonstrate severe pathological consequences like liver failure, cirrhosis and hepatocellular carcinoma.

Current genomic typing of HBV confirms the distribution of HBV into eight genotypes, A - H. Genotypes A, B, C and D have been suggested as the predominant genotypes in Malaysia in common with other Asian countries. The molecular protocols for HBV genotyping include commercial hybridization assays, multiplex-PCR, PCR-restriction fragment length polymorphism (RFLP) and immunoassays that uses monoclonal antibodies against the *pre-S2* epitope. Genotyping of HBV in this study was carried out by PCR-RFLP with *Ava 2* and *Sau 3AI* for the *pre-S* region, and *Tas I* and *Hinf I* for the S-region. Samples with atypical restriction patterns were further analyzed using genotype-specific primer-PCR (GSP-PCR). The study population consisted of 115 randomly selected chronic hepatitis B patients positive for HBV DNA by the dot-blot hybridization assay.

The results obtained showed a frequency distribution of: genotype B - 53%, genotype C - 40%, genotype D - 1.7%, genotype A - 0.9% and indeterminants - 4.3%. Single genotype infection was observed in 95% (110/115) of the study population. Simultaneous infections by different subtypes of HBV were observed in 40% (46/115) of the individuals with the most frequent subtype combination being B1/B5 at 70% (32/46). Genotyping of the *Pre-S* region by PCR-RFLP indicated the presence of deletions in HBV genotypes A, B and C.

In conclusion, the PCR-RFLP assay established provides a rapid protocol as it only requires a single amplification reaction set-up. In addition, the PCR-RFLP assay allows the identification of HBV subtypes and detection of deletion mutants.

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Mitochondrial mutations of Lebers hereditary optic neuropathy: a risk factor for multiple sclerosis? *R. Cittadella, I. Manna, A. La Russa, M. Liguori, V. Andreoli.* Institute of Neurological Sciences,National Resarch Council,Piano Lago of Mangone, Cosenza, Italy.

The occurrence of optic neuropathy in patients with MS-like disorders who carry one of the pathogenically significant mutations of Lebers hereditary optic neuropathy (LHON), a maternally transmitted disease characterized by acute or subacute, painless, bilateral loss of central vision due to optic nerve atrophy, as well as preferential maternal transmission of MS in affected parent-child pairs sopport the hypothesis that mitochondrial genes may implicate the etiology of MS. To evaluate the link between MS and LHON primary point mutations, we investigated a cohort of non related Italian clinically definite MS patients with optic nerve atrophy, as well as a cohort of patients without involvement of the optic nerve as controls. Each subject recruited to the study was fully informed and gave his/her consent. We searched for the presence of LHON mitochondrial mutations at nucleotide positions (np) 11,778, 3,460, 14,484 and 15,257 by mutation-specific polymerase chain reaction and restriction fragment length polymorphism. Our results suggest that there is no association between Italian patients with MS and mtDNA point mutations at np 11,778, 3,460, 14,484 and 15,257. Meanwhile, the results of several studies suggest a multifactorial etiology for MS, including multiple genetic factors of moderate effect rather than a very few genes of major biologic importance. However, the hypothesis that mitochondrial genes may implicate susceptibility to MS cannot be excluded.

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Functional prove and further clinical evidence of the pathogenic relevance of TBX1 missense mutations. *A. Rauch[1], C. Zweier[1], H. Sticht[2], C. Campbell[3].* 1) Institute of Human Genetics, Friedrich-Alexander Univ, Erlangen, Germany; 2) Department of Bioinformatics of the Institute of Biochemistry, Friedrich-Alexander Univ, Erlangen, Germany; 3) Department of Biochemistry, University at Buffalo, NY.

Deletion 22q11.2 syndrome is the most frequent known microdeletion syndrome. It is associated with a highly variable phenotype including DiGeorge- and Shprintzen syndromes. From studies in several mouse models, haploinsufficiency of the T-box transcription factor TBX1, which is located within the common deletion interval, was suggested to cause the phenotype. Nevertheless, to date only 3 patients from Japan were described to have point mutations of TBX1 in association with five of the major phenotypes of 22q11.2 deletion. We report the first Caucasian patient with a TBX1 missense mutation within the T-box, associated with the typical facial gestalt, short stature and developmental delay. While all features are known to be associated with the 22q11.2 deletion syndrome, short stature and developmental delay were not previously reported in individuals with TBX1 mutations. The mutation in our patient was inherited from his father, who also showed short stature and facial anomalies. To prove the functional relevance of this mutation we tested our novel and the three published mutations in a transcriptional reporter assay. While the published truncating mutation showed remarkable reduction of TBX1 transcriptional activity, the published and our novel missense mutation showed significantly increased activity. The latter finding is in accordance with data from mouse models, showing the same phenotypic spectrum in both, TBX1 under- and overexpression. We therefore provide the first functional evidence for the pathogenic relevance of TBX1 missense mutations.

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A locus for a new recessive form of limb-girdle muscular dystrophy with quadriceps atrophy. *J. Jarry[1], M.F. Rioux[1], Y. Robitaille[2], V. Khoury[3], I. Thiffault[1], M. Tétreault[1], L. Loisel[1], J.P. Bouchard[4], B. Brais[1].* 1) Laboratoire de neurogénétique, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Québec Canada; 2) Hôpital Ste-Justine, Montréal, Québec, Canada; 3) Département de radiologie, Hôpital Notre-Dame, Montréal, Québec, Canada; 4) CHA-Hôpital Enfant-Jésus, Université Laval, Québec, Canada.

Limb-girdle muscular dystrophies (LGMD) are characterized by a weakness and wasting of the proximal limb muscles, with typical sparing of the facial muscles. So far, seven autosomal dominant forms (AD-LGMD) and ten autosomal recessive forms (AR-LGMD) have been characterized. We have identified a cohort of 14 patients belonging to eight French-Canadian families displaying a distinct autosomal recessive form of limb-girdle muscular dystrophy with biceps and quadriceps atrophy and myalgia. This new form of LGMD displays extreme intra- and interfamilial variability. Clinical examination, magnetic resonance imaging, electromyograms, and muscle biopsies were performed to confirm the LGMD phenotype and to differentiate it from previously-described forms of the disease. A genome-wide scan was done with affected and unaffected individuals from two informative families and linkage was found to a less-than-3.3-cM locus not known to be linked to an LGMD (multipoint LOD score 4.56). We propose to name this new form LGMD2K.

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Identification of a Novel Lamin A/C Mutation in a Chinese Family Affected with Prominent Atrioventricular Block and Autosomal Dominant Dilated Cardiomyopathy. *Q. Wang[1,2], L. Gui[2,3], X. Zhang[2], M. Liu[2], W. Li[2,3], L. Yan[4], R. Jin[3], Q. Wang[2,3], T. Ke[2], X. Wang[2], Zh. Tang[2], J. Yang[2,3], X. Wu[2,3].* 1) Ctr Molecular Genetics, ND40, Cleveland Clinic Foundation, Cleveland, OH; 2) Huazhong University of Science and Technology Center for Human Genome Research, Wuhan, Hubei 430074, China; 3) Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, China; 4) Electrocardiogram Lab, Jinshan Hospital, Jinshan, Shandong, 256000, China.

Abstract Dilated cardiomyopathy (DCM) is a disorder characterized by cardiac dilation and reduced systolic function. About 20% to 25% of DCM cases are familial, and the LMNA gene encoding the nuclear envelope protein lamin A/C is the most common gene identified for familial DCM. In this study, we characterized a large Chinese family with atrioventricular block (AVB) as the most prominent feature. Some patients in the family also have later onset DCM. No skeletal muscle dysfunction or joint contractures were detected in any member of the family. Genomewide linkage analysis mapped the AVB gene in the family to a marker at chromosome 1q21.2, where the LMNA gene is located. Direct DNA sequence analysis revealed a heterozygous G to A transition at nucleotide 244 in exon 1 of LMNA (GenBank accession number, 4000), resulting in a novel E82K mutation. The E82K mutation co-segregated with all affected individuals in the family, and is not present in 200 normal controls. Mutation E82K is located in the coil 1B rod domain of lamins A/C, which is a highly conserved unique heptad repeat domain specific to nuclear lamins . Conduction system disease in the family began in the third decades, and DCM developed in the fourth and fifth decades and is accompanied by advanced AV block. Echocardiography showed that atrial dilatation occurred earlier than ventricular dilatation in the patients. Ejection fractions (EF) didnt decrease even in the patients with the third degree AVB and AF. Our results identify the first, novel LMNA mutation in the Chinese population, and suggest that defects in lamin A/C plays an important role in families with AVB as the prominent clinical feature.

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Myotilin mutations are associated with a significant number of Myofibrillar myopathy cases. *A. Shatunov[1], M. Olive[2], D. Hilton-Jones[3], I. Ferrer[2], M.C. Dalakas[1], L.G. Goldfarb[1].* 1) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA; 2) IDIBELL-Hospital de Bellvitge, Institut de Neurolpatologia, Barselona, Spain; 3) Department of Clinical Neurology, Radcliffe Infirmary, Oxford, United Kingdom.

Mutations in the Myotilin (MYOT, TTID) gene have been associated with variable syndromes including Limb girdle muscular dystrophy type 1A (LGMD1A) and a subgroup of Myofibrillar myopathy (MFM). We tested for MYOT mutations patients diagnosed as MFM, Desmin-related myopathy or Desminopathy, characterized clinically by lower limb muscle weakness slowly spreading to involve truncal, neck-flexor, facial, bulbar and respiratory muscles; skeletal myopathy was often combined with cardiomyopathy manifested by conduction blocks and arrhythmias. Sections of the affected skeletal and cardiac muscles showed abnormal fiber areas containing amorphous desmin-immunopositive deposits. Of a multinational MFM cohort consisting of 122 affected families or sporadic patients, eleven (9%) were identified as carrying MYOT mutations. Of these, Ser55Phe, Ser60Cys and Ser60Phe mutations were previously described, and Lys36Glu and Gln74Lys were novel. To compare, 24 of these families (20%) show the presence of mutations in desmin and none was found to carry mutations in alphaB-crystallin. The MYOT mutation-associated cases presented at the age of 42 to 77 years with lower limb muscle weakness and associated signs of cardiomyopathy, respiratory failure and peripheral neuropathy. Myopathological features of patients with MYOT mutations differed from desminopathy in several aspects: they exhibited (a) more abundant plaque-like irregular polymorphous inclusions strongly immunoreactive to myotilin, in addition to desmin; (b) multiple and prominent rimmed and centrally or subsarcolemmally located non-rimmed vacuoles; and (c) at the ultrastructural level, streaming Z-lines and focal dissolution of myofibrils at the absence of granulofilamentous material typically observed in phenotypes associated with desmin mutations. The frequency of Myotilinopathy in Spain was much higher than in other populations reaching 60% of cases diagnosed as MFM.

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The clinical impact of emerging Dystrophin gene mutation detection techniques. *M.F. Buckley[1], P.J. Taylor[1], R. Pedersen[2], G.L. Mullan[1], S.L. Maroulis[1], B. Prosper Gutiérrez[1], F. de la Puente Alonso[1], H. Johnston[3], D. Mowat[1].* 1) Molecular & Cytogenetics Unit, Prince of Wales Hosp, Sydney, Australia; 2) Department of Medical Genetics, Sydney Children's Hosp, Randwick, Australia; 3) Department of Neurology, Sydney Children's Hosp, Randwick, Australia.

Mutation detection for the DMD gene is complicated by the fact that it is a large gene consisting of 79 exons spread over 2.2 MBp of gDNA. Most mutations involve copy number changes of one or more exons. Here we report the evolution, over the past twenty years, of a clinical diagnostic testing program for Duchenne and Becker muscular dystrophy. We examine both the changes in the observed mutation spectrum and the clinical impact of the improved capacity to detect exon deletions and duplications in a cohort of 512 Australian families referred to our laboratory. The three techniques considered are multiplex exon PCR, semi-quantitative PCR and MLPA. 40 percent of B/DMD families had a deletion mutation detected in an affected male individual by a multiplex PCR approach involving the analysis of 25 exons. Semi-quantitative PCR of 19 exons detected a further 4 percent deletion mutations in females who were either manifesting carriers of DMD or relatives of an affected male for which no DNA was available for testing. Thus, had DNA been available on an affected male individual for each family, the overall detection rate by exon multiplex PCR would have been 44 percent. MLPA of all 79 exons on patients in which a deletion was not detected by other methods increased the overall mutation detection rate for deletions to 50 percent. In 7 per cent of D/BMD families a duplication involving one or more exons was detected by semi-quantitative PCR of 19 exons with a further 4 percent being detected by MLPA. Overall, 11 percent of families were found to have exon duplications. In summary MLPA increased the combined detection rate of exon deletion and duplication mutations from 50 to 62 percent. In addition to this, MLPA inadvertently detected seven point mutations that lay under the probe binding sites.

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Relationship between SCN1A mutations and SMEI. *S. Carrideo[1], E. Mannarino[2], G. Tortorella[2], G. Incorpora[3], A. Polizzi[3], F. Annesi[1], A. Labate[1,4], E.V. De Marco[1], F.E. Rocca[1], I.C Cirò Candiano[1], D. Civitelli[1], P. Spadafora[1], P. Tarantino[1], A. Gambardella[1,4], G. Annesi[1].* 1) Intitute of Neurological Sciences, National Research Council, Piano Lago - Mangone (CS), Italy; 2) Department of Paediatrics, University of Messina, Italy; 3) Department of Paediatrics, University of Catania, Italy; 4) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Mutations in the SCN1A gene are the major causes of GEFS+, which shows a marked phenotypic heterogeneity within families and most commonly manifests as febrile seizures (FS), or FS plus. It still remains uncertain if severe myoclonic epilepsy in infancy (SMEI) represents the end of the spectrum within the GEFS+ phenotype, or it should be considered a distinct entity, that is often related to de novo frameshift and nonsense mutations. Here we report three novel SCN1A mutations in three different patients. Two patients had SMEI phenotype with early infantile febrile seizures,absences, cognitive impairment, ataxia and drug resistance. The third patient had a milder epilepsy phenotype and normal neurological development. He had early prolonged FS, afebrile motor seizures and absences, which disappeared with antiepileptic therapy. The 26 exons of SCN1A gene were individually amplified using primers based on intronic sequences. The PCR products were then sequenced and analyzed with an automatic sequencer. We identified three novel SCN1A mutations. In the first of the two SMEI patients we found a missense mutation, T1289I. His mother and brother carried the same mutation, but they never had any seizure. The second SMEI patient carried a de novo frameshift mutation, 3840insT, that lead to a premature stop codon in the exon 19. The third patient with a milder phenotype carried a de novo single nucleotide substitution in the invariant AG splice acceptor site of intron 24. The results of our study illustrate that de novo SCN1A mutations do not always mean SMEI and vice versa. In this way, our study reinforce the belief that SMEI probably results from the cumulative effects or interactions of a few or several genes, of which the reported GEFS+ gene is only one player.

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A novel exon skipping mutation of the NSD1 gene presents heterogeneous phenotypes of Sotos syndrome in a Japanese family. *S. Tei, S. Tsuneishi, M. Matsuo.* Pediatrics, Kobe University, Kobe, hyogo, Japan.

Sotos syndrome (SoS) is characterized by pre- and post-natal overgrowth, advanced bone age, distinctive craniofacial features (macrocephaly, frontal bossing with high hairline, high palate, prominent jaw), and variable degrees of mental retardation and is caused by mutations of the NSD1 gene located in 5q35. Microdeletions encompassing of this gene are identified in more than half of the Japanese cases. SoS with microdeletions shows often severer phenotypes than those carrying point mutations. Only 5 familial cases of SoS have been reported with confirmation of gene analysis. Here we report a Japanese family with SoS showing heterogeneous phenotypes and a novel mutation of the NSD1 gene in every affected member. We found the initial case (first son) from unconsanguineous parents who had typical phenotypes of SoS and died of Reye syndrome. Two consecutive children also met the criteria of SoS. Genetical analysis confirmed the first Japanese familial cases (mother and 3 children) carrying the same splice donor site mutation (IVS13+1G>A), which results in the in-frame skipping of exon 13. However, each case shows variable phenotypes and severity. Male individuals (two sons) showed symptoms related with central nervous system (CNS) including seizures, severer mental delay, feeding difficulty in infancy and periventricular subependymal cysts. Female individuals (daughter and mother) showed milder mental delay, and the daughter carried congenital heart defect (pulmonary stenosis). There may exist some difference dependent on gender in NSD1 splicing and/or epigenetic pathogenesis especially in CNS.

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PTPN11 mutations in Noonan Syndrome: Mutation detection and phenotypic variability. *M. Hegde, J. Wiszniewska, A. Cirigliano, P. Ward, C. Eng, B. Roa.* Medical Genetics Laboratories, Baylor Col Medicine, Houston, TX.

Noonan syndrome (NS) is an autosomal dominant disorder characterized by a spectrum of symptoms and physical features.Affected individuals present with short stature,dysmorphic features,pulmonary valve stenosis and hypertrophic cardiomyopathy.NS is genetically heterogeneous,with mutations in the PTPN11 gene accounting for ~50% of both familial and sporadic cases.During the two- year period ending June 2005,280 individuals were referred to our laboratory for PTPN11 gene sequencing.In 113 individuals (40%)PTPN11 missense mutations were identified.We present data on two families with a striking variation in phenotypic expression for two allelic missense variants that affect PTPN11 amino acid residue Q510. The Q510 residue occurs in the phosphotyrosine phosphatase (PTP) domain of the PTPN11 protein and is evolutionary conserved. Allelic missense mutations Q510E and Q510P have been reported in a case of Noonan syndrome and in LEOPARD families, respectively.Notably, in one reported LEOPARD family, the Q510P mutation was found in an affected child and also in the mother,who did not present with clinical features except for lentigines.We studied a 3-yr old patient with clinical findings suggestive of Noonan syndrome,and identified the (1529A>C) Q510P heterozygous missense allele. Subsequent analysis of family members identified the Q510P in the patients mother and one sibling who both have similar findings as the proband;in addition, Q510P was also found in another sibling who is reported to be unaffected.A second family demonstrates clinical heterogeneity of the Q510R missense variant,which has not been previously reported.In this family, sequence analysis identified the (1529A>G) Q510R missense variant in a 41 yr old woman diagnosed with NS. Subsequent analysis identified the Q510R in one affected child, but not in a second child who is also reported to be affected.Allelic amino acid substitutions involving Q510 highlight the allelic and clinical heterogeneity of PTPN11 gene mutations associated with a spectrum of clinical presentations between NS and LEOPARD syndromes.These data highlight difficulties in genotype:phenotype correlations involving the PTPN11gene.

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SPG7 analysis in spastic paraplegia: rare causative mutations but frequent variations. *N. Elleuch[1], C. Depienne[1], A. Benomar[2], AM. Ouvrard Hernandez[3], X. Ferrer[4], B. Fontaine[5], D. Grid[6], C.ME. Tallaksen[1], R. Zemmouri[7], G. Stevanin[1], A. Durr[1], A. Brice[1].* 1) INSERM U679 et Département de Génétique Cytogénétique et Embryologie, Hôpital de la Pitié-Salpêtrière, Paris, France; 2) Laboratory of Neurogenetics and the Department of Neurology, Hôpital des Spécialités, Rabat, Morocco; 3) Department of Neurology. Centre Hospitalier Universitaire, Grenoble, France; 4) Department of Neurology. Centre Hospitalier Universitaire, Bordeaux, France; 5) INSERM U546, Hôpital de la Pitié-Salpêtrière, Paris, France; 6) Généthon III, Evry, France; 7) Department of Neurology, Centre Hospitalier Universitaire Mustapha Bacha, Place du 1er Mai, 16000, Alger, Algeria.

Mutations in the SPG7 gene, which encodes paraplegin, are responsible for an autosomal recessive (AR) form of pure or complex hereditary spastic paraplegia (HSP). The aim of our study was to evaluate the involvement of the SPG7 gene in a large population of HSP families compatible with AR transmission. We analysed 136 probands with pure or complex HSP for mutations in the SPG7 using DHPLC and/or direct sequencing. We identified 49 variants including 8 mutations, 29 polymorphisms and 12 changes without known effect. In two families, originating from Morocco and Mauritania, we confirmed the deleterious nature of the compound heterozygous mutations. Both had complex HSP with cerebellar impairment. The ages at onset were similar, ranging from 27 to 32 years, and progression of the disease was rapid in both families. We also detected 19 families with only one mutation that was not found in a large control population. The mutations were highly deleterious in 4 of these families (3%), suggesting that they were probably causative. No mutation on the other allele was detected by direct sequencing of all exons or RT-PCR experiments. Our results therefore indicate that SPG7 mutations are rare and account for approximately 5% of HSP families compatible with AR inheritance. Cerebellar signs, or cerebellar atrophy on brain imaging, were the most frequent additional features in patients with SPG7 HSP. Finally, rare nucleotid variants in SPG7 are very frequent, complicating routine diagnosis.

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The natural history of arrhythmogenic right ventricular cardiomyopathy (ARVC) in a homogeneous Newfoundland population. *T. Young[1], P. Parfrey,[2], S. Connors,[3], L. Thierfelder,[4], A. Bassett,[5], K. Hodgkinson[1,2].* 1) Discipline of Genetics, Memorial University, St John's, NF, Canada; 2) Patient Research Centre,Memorial University, St John's, NF, Canada; 3) Division of Cardiology, Memorial University, St John's, NF, Canada; 4) Max-Delbruk Centre, Berlin, Germany; 5) Clinical Genetics Research Program, CAMH, Toronto, ON, Canada.

Background: The natural history of ARVC in an etiologically homogeneous group is unknown. **Objectives**: To assess the natural history of autosomal dominant ARVC in a founder population linked to a 2cM DNA haplotype at 3p25 (ARVD5. **Methods**: We studied 426 subjects from well ascertained sibships (where the disease status of 50% of the sibship is known) from 16 ARVC families born at 50% a priori pedigree risk. Disease status was defined as high-risk (HR), low-risk (LR) or unknown based on serious cardiac events before 50 years, DNA haplotype testing and pedigree position. Subjects were ascertained both retrospectively (from the pedigree) and prospectively (from a screening clinic). We compared both male and female HR and LR subjects on age at presyncope (PS), syncope (S), palpitations (P), chest pain (CP), heart failure (CHF), hospitalization for cardiac events (H) and death. For the death analysis we used subjects from sibships where 100% of siblings disease status was known, and where HR status was based on DNA haplotype or pedigree position. **Results**: Time to event was significantly younger for HR (n=79) than LR (n=53) males for PS, P, CHF, H and death (p values 0.008 to 0.0001). The majority (75%) of male deaths were sudden. Significant differences were restricted to S and P (p=0.02) for HR (n=74) and LR (n=48) females. CHF, H and death occurred at significantly younger ages for HR males than HR females (p= 0.0001). **Conclusions**: Sudden cardiac death occurs frequently in males with heart failure a later manifestation in survivors. Chest pain appears not to be associated with disease. Palpitations may be useful diagnostic clues for both sexes.

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Patients Learning About the Natural Evolution of Tuberous Sclerosis (PLANETS) Study. I: Description of the cohort and results for kidney angiomyolipomas. *B.E. Gould Rothberg, M.B. Bracken, E.B. Claus.* Department of Epidemiology and Public Health,Yale University, New Haven, CT.

Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder caused by mutation in either the tuberin (TSC2) or hamartin (TSC1) genes. Although TSC is nearly 100% penetrant, its highly variable expressivity has hampered TSC natural history evaluations. To date, population-based epidemiologic studies performed have been underpowered. Here we describe the Patients Learning About the Natural Evolution of Tuberous Sclerosis (PLANETS) Study, our effort to assemble a large TSC patient cohort. We created a 268-item questionnaire addressing the major and minor TSC clinical manifestations, TSC genetics, patient demographics and general medical history. During Fall 2004, 7598 questionnaires were mailed to TS Alliance constituents recorded as TSC patients or their parents/grandparents/caregivers. Through February 2005, 938 surveys were completed and returned; 198 (21.1%) were self-completed with the remaining 740 (78.9%) by proxy. The mean age for the entire population is 21.6 15.9 years and 508 (54.2%) of the respondents are female. By gender, the mean age for females is 23.4 16.5 years and, for males, 19.4 14.9 years ($p<0.001$). 839 (90.1%) self-identified as White non-Hispanic with the remaining 99 listing another race. We selected kidney angiomyolipomas (AMLs) as the prototype TSC-related symptom to pilot our analysis. Of the 836 patients who underwent kidney imaging, 376 (45.0%) reported having kidney AMLs, estimating the prevalence of AMLs between 42% and 48%. Multivariable logistic regression reveals a 1.68-fold (95% CI=1.24-2.24) increased prevalence of AMLs in females versus males. We defined the cumulative incidence of kidney AMLs for subjects born since 1990 and report the median age at initial AML diagnosis as between 8-12 years. 112 (32%) of participants with AMLs reported either an AML hemorrhage or an AML-reducing procedure. These data establish PLANETS as the largest cohort of TSC patients ever assembled where extensive phenotypic annotation is available and will allow for the description of Tuberous Sclerosis Complex natural history.

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Low concentrations of 25-hydroxy-vitamin D in sera of people with neurofibromatosis 1. *V. Mautner[1], M. Lammert[1], R. Friedrich[1], L. Kluwe[1], P. Lim[1], J.M. Friedman[2].* 1) Dept Neurology, Allgemeines Krank Ochsenzoll, Hamburg, Germany; 2) Dept Medical Genetics, University of British Columbia, Vancouver, Canada.

Osseous involvement is one of the cardinal features of neurofibromatosis 1 (NF1). The most characteristic bony manifestations are considered to be focal dysplastic lesions, but short stature is often seen and recent studies show that decreased bone mineral density frequently occurs in people with NF1. This is an important cause of morbidity, mortality and poor quality of life in individuals with NF1, yet the pathophysiology is not understood. Acquisition and/or synthesis and metabolism of vitamin D is one possible mechanism that influences bone mineralization. The purpose of this study is to determine if serum levels of 25-hydroxy vitamin D (25-OHD) in people with NF1 differ significantly from levels in controls.

We compared 25-OHD levels in sera of 53 individuals with NF1 to 58 controls. People with evidence of chronic liver disease, epilepsy, chronic GI disease, vitamin D supplementation, or unusual UV light exposure were excluded from the analysis. The mean value for 25-OHD in NF1 patients was 15.7 ug/l (sd=7.5) compared to 35.5 (sd=17.1) for controls ($p<<.001$). Gender ratios were similar in the groups: 33/53 NF1 patients and 38/58 controls were female. Mean ages of patients (39.3, sd=9.6) and controls (36.0, sd=8.8) were comparable, as was the time of year in which the sera were collected, the majority being collected in the winter months. Serum calcium levels on an unselected subset of the NF1 patients are similar between NF1 and control groups: NF1 mean level is 2.39mmol/L (n=18) and control mean is 2.38 mmol/L (n=58).

Further analysis will investigate other possible aspects of bone metabolism in NF1 patients and the influence of vitamin D on bone metabolism and calcium homeostasis. Vitamin D plays a key role in bone mineralization and low levels may provide an explanation for the osteopenia seen in NF1 and might offer the promise of a simple and inexpensive preventative strategy.

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Defining phenotypic subgroups in autism using the Aberrant Behavior Checklist-C. *J.S. Brinkley[1], S. Donnelly[1], H. Cope[1], C. Wolpert[1], S. Brinkley[1], R. Carney[1], H. Wright[2], A. Hall[2], R. Abramson[2], J. Gilbert[1], M. Pericak-Vance[1], M. Cuccaro[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Dept of Neuropsychiatry, USC-SOM, Columbia, SC.

Efforts to identify susceptibility genes in autism have been hampered by genetic heterogeneity. Given the wide clinical variation in autism, a common strategy to address heterogeneity is use of clinically derived subgroups. These subgroups are then analyzed to determine whether the shared clinical features reflect underlying genetic mechanisms. In a novel approach to subgroup development, we employed the Aberrant Behavior Checklist-Community (ABC-C), a 58-item rating scale of behavior problems in individuals with developmental disorders. Using dimension reduction methods in our autism sample (N=276, M=9.4 years, sd=4.9) we identified two problem behavior factors from the ABC-C/ These factors are based on severity of self-injurious behavior (SIB) and intensity of verbally disruptive behavior (VSD) and created subgroups for each factor. These factors are not correlated (r=.23) and subgroups based on these factors differed significantly on multiple clinical indicators. Comparison of multiplex and singleton probands on the SIB and VSD mean factor scores revealed no differences on the VSD factor (p=0.80) but the multiplex group had significantly higher mean SIB scores (t=1.98, p=0.048). Intra-family correlations in 73 multiplex families yielded low concordance (SIB ICC= .15; VSD ICC = .06). Stratified analyses were conducted for regions of interest on chromosomes 15 and 17. Two-point linkage analysis (FASTLINK) yielded a 2-point Max HLOD of 1.69 for a HCV8865209 under the recessive model for the stratified SIB+ group. No other positive linkage results were found for SIB and VSD in stratified analyses. Similarly, no allelic or genotype association was detected using the pedigree disequilibrium test (PDT) and GenoPDT for multiple SNPs on chromosomes 15 and 17. In conclusion, both SIB and VSD appear to index distinct clinical groups within our autism dataset. Preliminary findings suggest that these phenotypic subgroups are not the result of underlying genetic mechanisms.

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Autism in African American (AA) families: Phenotypic findings. *M. Cuccaro[1], S. Donnelly[1], H. Cope[1], C. Wolpert[1], R. Carney[1], R. Abramson[2], A. Hall[2], H. Wright[2], J. Gilbert[1], M. Pericak-Vance[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Dpt of Neuropsychiatry, USC-SOM, Columbia, SC.

Genetic studies of autism, a common complex disease, encourage participants from all racial groups, but AA participants are underrepresented. Despite evidence that phenotypic and genetic differences exist in AA individuals in complex diseases such as Alzheimer disease and schizophrenia, there has been limited study of phenotypic variation in autism. Finally, study of AA families with autism fulfills a national scientific priority to involve minority populations in health related research. This study represents the first effort to systematically compare AA individuals with autism on standard phenotypic measures. Participants (AA N= 41/CA N=254) were ascertained through an autism genetics study. The clinical protocol included the ADI-R, Vineland Adaptive Behavior Scales (VABS), and Aberrant Behavior Checklist (ABC). The groups were compared on ADI-R items (initial words, phrase speech, motor coordination and age of onset), VABS Adaptive Composite Score and ABC scales. AA participants had delayed acquisition of first words (2=13.32, df=2, p<0.001) and phrase speech (2=10.33, df=2, p <0.006) and borderline significantly lower VABS composite scores (t=1.92, df=227, p=0.057). The groups did not differ on motor coordination, age of onset, or ABC scales. Our findings reveal phenotypic differences in AA autism families. Specifically, clinical-phenotypic differences indicative of a more severe phenotype were found in the AA participants. While we cannot rule out an ascertainment bias, the groups only differed on select measures. We conclude that efforts to track genetic variations in autism consider increased recruiting of different ethnic groups. Such can efforts can help identify potential phenotypic differences which may index unique genetic variation that confers risk to autism. Preliminary investigation of GABAergic candidates in AA families by our group (Collins et al, this meeting) suggests that phenotypic differences may not reflect underlying genetic susceptibility. However, there may be other loci that modify the susceptibility phenotype.

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Behavioral phenotype of Cornelia de Lange syndrome: Relationship to physical phenotype, age and parenting stress. *C. Landy[1], B. Clark[2], A. Kline[3], M. Grados[4].* 1) University of Maryland, Baltimore, MD; 2) Johns Hopkins University, Baltimore, MD; 3) Greater Baltimore Medical Center, Baltimore, MD; 4) Johns Hopkins School of Medicine, Baltimore, MD.

Background: Cornelia de Lange syndrome (CdLS) is a generally sporadic multiple malformation syndrome involving characteristic facial features, growth retardation and mental retardation, for which the causal gene NIPB-L (5p13.1, 608667) has been recently identified. Behaviors associated with CDLS include self-injurious behavior (SIB), hyperactivity, aggression and stereotyped movements. The purpose of the present study was to characterize maladaptive behaviors in relation to physical features and examine the longitudinal manifestation of maladaptive behaviors in CdLS. The ultimate goal of this research is to define a behavioral phenotype of CdLS. Methods: Data was obtained from the parents of 28 affected children, ages 5-17 years, through mailed surveys and telephone interviews. The Aberrant Behavior Checklist (ABC) measured aggression, SIB, irritability, hyperactivity, and stereotypy. The Scoring System for Severity in Cornelia de Lange Syndrome determined severity of the physical manifestations of the disorder, and the Parental Stress Index (PSI) measured stress levels in the parents. Results: The mean age was 11 3.74 years, 39% male and 61% female. A significant positive relationship was seen between minor facial/physical features and hyperactivity ($t=2.54$, $p<.05$) and stereotypy ($t=2.27$, $p<.05$). Among physical features, the association was most notable for 2-3 toe syndactyly ($p < 0.01$). SIB was significantly higher in participants > 12 years ($t=2.20$, $p<.05$). Hyperactivity and stereotypies were not age-related. Parents of children with more severe behaviors showed marginally greater role restriction ($0.05 < p < 0.10$). Conclusions: Hyperactivity and stereotypy in children with CdLS appear to be associated with physical features of the disorder while SIB manifests at older ages. The severity of behaviors in CDLS may determine parental role restriction.

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A fourth case further delineates the phenotype of a rare karyotype abnormality: 46,XX/47,XX,+r(X). *K. Jenny[1], I. Gadi[2], JJD. Morrissette[3], B. Powell[4], KW. Gripp[1].* 1) AI duPont Hosp for Children, Wilmington, DE; 2) Labcorp, Research Triangle Park, NC; 3) St Christopher's Hosp for Children, Phila, PA; 4) Children's Hosp Central, Madera, CA.

Introduction: Small supernumerary marker chromosomes (sSMC) occur in ~3/10,000 live births. The risk for an abnormal phenotype is 10-15%. About 60% of sSMCs are derivative chromosome 15; Pallister-Killian and Cat-Eye syndromes are due to extra derivative chromosomes 12 and 22, respectively. The sSMCs are classified as satellites or nonsatellited and often occur as mosaic. Ring chromosomes account for ~10% of sSMC. Supernumerary r(X) are rare, particularly without a 45,X cell line. Tumer (1998) reported two females and Powell (1999) reported one, each mosaic for a cell line with a small supernumerary r(X). Phenotypes were similar with jaundice, poor feeding, hypotonia, seizures, developmental delay, obesity, minor dysmorphia including flat face, low nasal bridge, small mouth and clinodactyly. The pt reported by Powell has since died from pneumonia and seizures. **Case Report:** A female presented at 12 months with hypotonia, developmental delay, brachycephaly, and asymmetric white matter volume loss on brain MRI. Pregnancy was notable for reduced fetal activity. Neonatal course included torticollis, gastroesophageal reflux, and jaundice treated for several weeks. Developmental delay became obvious at 7 months with poor head control and inability to sit independently. Chromosomes revealed a marker ring chromosome in 100% of lymphocytes. FISH studies showed the ring to be a derivative X chromosome, lacking the XIST gene. Buccal smear confirmed the marker to be present in 40% of cells; 46,XX/47,XX,+r(X). **Discussion:** 3 females with supernumerary r(X) lacking XIST are reported in the literature. We report a fourth case of 46,XX/47,XX,+r(X). All had developmental delay, hypotonia, obesity and minor dysmorphia. The XIST gene is necessary for the initiation of cis-acting X inactivation, suggesting that the genes present on the r(X) are active, resulting in a functional X disomy for these genes. The phenotype shared amongst the four pts likely results from functional genes present on the r(X).

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The association of DAT1 gene Core Promoter polymorphism -67 T/A with ADHD. *A. Irani Shemirani[1,2], N. Moghimi[2], M. Keikhaee[2], E. Shirazi[3], M. Tehranidoost[4], S. Ehssani[2], H. najmabadi[2], M. Ohadi[2].* 1) Islamic Azad university,science and research branch,poonak, Tehran, Iran; 2) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Evin, Tehran, Iran; 3) Iran university of Medical Science, Tehran, Iran; 4) Department of Psychiatry, Rozbeh Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Association between attention deficit hyperactivity disorder (ADHD) and the 10-repeat allele of a polymorphism (a 40 bp variable number of tandem repeats) in the dopamine transporter gene (DAT1) has been widely documented. In this study, we examined whether either allele of the DAT1 core promoter -67 polymorphism is associated with ADHD in a case/control study. The allele and genotype frequencies of the polymorphism were studied in 100 patients and 163 controls, which were matched on the basis of sex, age and ethnicity. The genotype frequencies in the patients group were as follows: AA=15.5%; AT=71.8%; TT=12.6% vs. the genotype frequencies in the control group: AA=49%; AT=41.8%; TT=9.2% [2=31.11, df = 2, OR = 2.15 (95% CI=1.34-3.47, p 0.0001]. The T-allele of the -67A/T polymorphism revealed a ~1.6-fold excess in the patients group comparing with the controls (2=18.45, df = 1, p 0.001). For the first time, these findings provide tentative evidence of the contribution of the DAT1 gene core promoter polymorphism to the etiopathophysiology of ADHD at least in the Iranian population that we have studied. Replication studies of independent samples and family-based association studies are necessary to further evaluate the significance of our findings.

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The optineurin gene and apolipoprotein E gene polymorphism in Japanese patients with normal tension glaucoma and primary open-angle glaucoma. *F. Mabuchi[1], S. Tang[2], Y. Toda[1], D. Ando[2], M. Yamakita[2], J. Wang[2], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1].* 1) Dept Ophthalmology, Univ Yamanashi, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Yamanashi, Japan.

Objective: To assess whether genetic polymorphisms of the optineurin (OPTN) gene and the apolipoprotein E (APOE) gene are associated with normal tension glaucoma (NTG) and primary open-angle glaucoma (POAG) in the Japanese population. Methods: Genomic DNA was examined in 144 patients with NTG, 166 patients with POAG, and 179 control subjects. PCR-SSCP and subsequent sequence analysis were performed for genotyping the OPTN gene, and the APOE allele frequency (2, 3, and 4 alleles) were studied by PCR-RFLP. A logistic regression model was used to simultaneously study the effect of multiple variables when comparing these patients with control subjects. Results: Adjusted for age, gender, and intraocular pressure, an appropriate twofold increased risk of NTG was found with the A alleles in c.412GA (P 0.05, odds ratio [OR] 2.17, 95% confidence interval [CI] 1.09 to 4.31) and c.603TA (P 0.05, OR 2.17, 95% CI 1.03 to 4.56) polymorphisms in the OPTN gene. An appropriate fourfold reduction in NTG risk (P 0.05, OR 0.27, 95% CI 0.09 to 0.77) was found with the APOE 2 allele. No statistically significant gene interaction was found between the OPTN and APOE gene polymorphisms for NTG and POAG. Conclusion: The OPTN and APOE gene polymorphisms were associated with NTG in the Japanese population. Further studies in the other ethnic populations should be performed to elucidate the relationship between these genes and glaucoma, and the interaction between these genes.

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Peer relationships in Neurofibromatosis 1. *J. Reiter-Purtill[1], E.K. Schorry[1], R.J. Hopkin[1], K. Vannatta[1], A. Lovell[1], C. Gerhardt[1], B. Moore[2], R.B. Noll[3].* 1) Cincinnati Children's Hospital Medical Center; 2) Univ. of Texas, MD Anderson; 3) Univ. of Pittsburgh.

Limited data are available regarding the social function of children with NF1 from the perspective of peers. We examined peer relationships of school-aged children with NF1, using peer and teacher report, to better define potential social difficulties and to investigate the impact of disease severity. Fifty-nine children with NF1 and 59 age- and gender-matched classmates participated. Data collected in the classroom included a measure of social behavior, and 2 measures of social acceptance. Each child with NF was also ranked by a clinical geneticist using scales of Medical Severity, Cosmetic Severity, and Neurologic Severity for NF1 complications. Children with NF1 were described by peers as less popular, more withdrawn, and as having less leadership qualities compared to controls. They were also less likely to be chosen as a "best friend" and to have reciprocated friendships. Peers described them as less attractive and less athletically and academically skilled than controls. Despite being ranked by peers as less physically attractive, 91% were ranked by clinicians as having "minimal or mild" cosmetic effects of NF1. Teachers perceived children with NF1 as less popular and more withdrawn. Subsequent mediational analyses suggested that peer perceptions of appearance, academic competence, and athletic ability explained the pathway between NF1 and poor peer functioning.

Previous peer studies conducted in children with other chronic diseases without primary CNS involvement have indicated few peer problems. In contrast, the social problems for children with NF1 are similar to our findings for children surviving brain tumors. Poor peer relationships in NF1 may be related to a direct effect of the NF1 gene on neurologic functioning, indicating a need for interventions to assist children with social skills.

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Aspiration and airway obstruction in CHARGE syndrome. *R.J. Hopkin, D.R. White, B.K. Giambra, C.L. Daines, M.J. Rutter.* Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH.

CHARGE syndrome is a complex condition caused by mutations in the CHD7 gene. It is characterized by combinations of malformations including Colobomas, Heart malformations, choanal Atresia, Retardation, Genital anomalies, and Ear abnormalities including deafness. CHARGE syndrome is often associated with cranial nerve palsies and global neurologic deficits. Respiratory distress and aspiration have been reported as common complications. In fact, aspiration may be the leading cause of death in CHARGE syndrome. Nevertheless, little has been published on the nature of the airway disease associated with CHARGE. We report a study of 30 patients with CHARGE evaluated at a single center. 27 had formal airway assessment using microlaryngoscopy, bronchoscopy, FEES, and or video swallow. RESULTS: 20 patients required tracheostomy. 20 had airway obstruction excluding choanal atresia, 9 at multiple levels. Obstruction was due to pharyngomalacia including glossoptosis, laryngomalacia, subglottic stenosis, and tracheomalacia. 18 patients had clear evidence of chronic aspiration or bronchiectasis. An additional 6 had abnormal swallow that placed them at very high risk for aspiration. Only 2 patients had normal swallowing. 25 patients had gastrostomy tubes placed for feeding problems. Risk of aspiration appeared to be independent of anatomic malformations, but was often neurologic in etiology. CONCLUSION: These data indicate that airway abnormalities other than choanal atresia leading to obstruction are very common in CHARGE syndrome. Further, we found that aspiration is among the leading complications of this condition. The aspiration is often due to neurologic rather than structural abnormalities and therefore unlikely to be amenable to reconstructive surgery. These findings have important implications as protocols are developed for evaluation and management to improve the out come of CHARGE syndrome. We recommend formal airway and swallow evaluation as a routine part of the management for every child diagnosed with CHARGE syndrome regardless of presenting malformations even without signs of respiratory distress.

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Congenital Malformations associated with misoprostol exposition during pregnancy in Colombia: case series. *C. Lopez, R. Garcia, F. Suarez, C. Villegas, I. Zarante.* Instiuto de Genetica Humana, Pontiifcia Universidad Javeriana, Bogota D.C., Colombia.

Misoprostol is a prostaglandin E1 analogue with abortive properties of limited efficacy, multiple descriptions of congenital defects associated with infants exposed to misoprostol in utero are reported. We presented 5 cases of newborns with congenital malformations, in which the mothers consumed and used intravaginal misoprostol application in order to interrup the pregnancy. The most frequent defects associated with Misoprostol in this series are equinovarus clubfoot, VII pair cranial nerve affection, and terminal transverse limb defects. We report a new case of bladder exstrophy with imperforate anus and one case of sirenomelia. This cases increase the evidence between congenital malformations and misoprostol as a teratogenic agent.

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Decreased glutathione and antioxidant enzyme activities in cellular models of Machado-Joseph disease. *C.L Kuo[1], Y.C. Yu[2], M. Hsieh[2,5], C.S. Liu[1,3,4].* 1) Vascular & Genomic Res, Changhua Christian Hosp, Changhua, Taiwan; 2) Department of Biology, Tunghai University, Taichung, Taiwan; 3) Department of Neurology, Chung Shan Medical University and Chung Shan Medical University Hospital, Taichung, Taiwan; 4) Department of Neurology, Changhua Christian Hospital, Changhua, Taiwan; 5) Life Science Research Center, Tunghai University, Taichung, Taiwan.

Machado-Joseph disease(MJD) is an autosomal dominant spinocerebellar degeneration characterized by cerebellar ataxia and pyramidal signs associated in varying degrees with a dystonic-rigid extrapyramidal syndrome or peripheral amyotrophy as major neurologic signs. Unstable CAG trinucleotide repeat expansion in MJD gene has been identified as the pathologic mutation of MJD. The nature of the toxic insult of the mutant proteins will cause aggregation in the nucleus and cytoplasma and eventually lead to cell apoptosis. It is known that oxidative stress, induced by reactive oxygen species (ROS) or free radicals, plays an important role in pathogenesis of neurodegenerative disorders. Nonenzymatic and enzymatic components in the antioxidative system play critical role(s) to against oxidative stresses in the mammalian central nervous system. In addition, both SK-N-SH-MJD78 and COS-7-MJD78 -GFP cell lines have the lower levels of catalase, glutathione reductase and superoxide dismutase when compared to the wild-type cell lines under normal growth condition. Furthermore, the expression of glutathione reductase decreased in COS-7-MJD78-GFP mutant cells compared to that of COS-7-MJD26-GFP normal cells. It is known that when cells are under oxidative stress, the mitochondrial DNA is prone to damage. Our results further demonstrated that mitochondrial DNA copy number of mutant cells is reduced when compared to that of the parental cells. Our results indicated that mutant ataxin-3 might influence the activity of enzymatic components to remove O2- and H2O2 efficiently, and promote mitochondrial DNA mutation or deletion, which leads to dysfunction of mitochondria. Therefore, we suggested that the cell damage caused by greater oxidative stress in SCA3 mutant cells plays an important role, at least in part, in the cell apoptotic pathway.

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Triallelic FMR1 gene in a girl with developmental delay and hyperkinetic behaviour. *J. Kapalanga[1,2,3], D.C. Riedell[3,4], A. Gandy[2,3], D. Wong[2,3].* 1) PEI Health Research Inst/SMC, Summerside, PE, Canada; 2) Pediatrics, Summerside Medical Center, Summerside, PEI, Canada;; 3) Faculty of Medicine, Dalhousie University, Halifax, NS, Canada; 4) Department of Pathology, Laboratory Medicine and Molecular Diagnostics, IWK Health Center, Halifax, Canada.

CGG trinucleotide repeat expansion in the 5end of the FMR1 gene is associated with fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and FMR1-related premature ovarian failure. Finding of more than two copies of normal-sized CGG repeats in association with a clinical syndrome has not been reported previously. Here we report a case with 3 distinct FMR1 alleles. The patient was a 5-year girl with developmental delay, hyponasal speech, hyperkinetic behaviour and short attention span. There is a history of developmental delay in several maternal male cousins. The patients weight and height were both above the 95th percentile and her head circumference was between the 90th to 95th percentile. Audiology evaluation was normal. Biochemical testing was negative. MRI was unremarkable. Her cytogenetic analysis was unrevealing. Fragile-X testing by PCR revealed normal sized FMR1 alleles. However, she carried 3 copies of the CCG repeat in the FMR1 gene, and had 3 distinct FMR1 alleles rather than the expected 2 alleles. Her allele sizes were 10, 24 and 30 CCG repeats. The mother was homozygous for a 30 trinucleotide repeat allele. Southern analysis showed normal banding patterns. However, careful examination of the Southern blot suggested that there were 2 copies of methylated alleles rather than 1 copy. These combined results indicate that, although she does not have a detectable expansion in her FMR1 genes, she does appear to have 3 copies of this genetic region. A possible explanation could be that she has a cryptic duplication within the Xq28 region, including at a minimum a portion of the FMR1 gene. So although she does not have an expansion in her FMR1 genes, it is not possible to rule out the possibility that changes in one of her FMR1 genes (or an adjacent region) might be the basis for her clinical symptoms. Other molecular testing strategies will be initiated to further analyze this mutation.

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Anophthalmia/Microphthalmia Registry: Registry clinical data support extended work-up of individuals with anophthalmia/microphthalmia. *A. Schneider[1], D. Moguillansky[2], T. Bardakjian[1].* 1) Dept of Genetics, Albert Einstein Medical Ctr, Philadelphia, PA; 2) Dept of Pediatrics, Albert Einstein Medical Ctr, Philadelphia, PA.

The Anophthalmia/Microphthalmia (A/M) Registry at Albert Einstein Medical Center (AEMC) in Philadelphia was established in 1994. Microphthalmia is a rare birth defect which is on the spectrum of eye anomalies with coloboma at the milder end and anophthalmia at the most severe end. Collectively the eye defect is referred to as MAC (microphthalmia, anophthalmia, coloboma) with an incidence of about 2/10,000 births. MAC is both clinically and genetically heterogeneous. There are well-described syndromes associated with MAC. However, gene screening is broadening the phenotypic spectrum of these conditions and at times merging previously distinct entities. The data collected thus far regarding the incidence of associated birth defects in the A/M Clinical Registry can be used to establish basic studies that should be performed on all individuals with MAC, whether or not a syndromal diagnosis is made. The data will also facilitate identification of new syndromes. Analysis of 200 cases in the A/M Registry revealed 104 males and 96 females. Overall the incidence of congenital heart disease in the Registry population was 11% compared to the general population incidence of less than 1%. Other striking findings included a high incidence of hearing loss, 4.5% compared to the population incidence of 0.3-0.4%, autism in 3.5% individuals compared to 0.2-0.6% in the general population and developmental delay in 34%. An in-depth analysis of this population will be presented. Our findings underscore the importance of a Clinical Genetics evaluation for every child with MAC and the necessity of a comprehensive work-up since not all findings are obvious. This should include a renal ultrasound, echocardiogram, hearing evaluation and developmental monitoring. The extended evaluation may help direct the gene screening to a specific gene or make a syndromal diagnosis which will provide the family with a prognosis and an accurate recurrence risk.

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A novel mutation in the GUCY2D gene responsible for an early-onset severe RP different from the usual GUCY2D-LCA phenotype. *S. Hanein[1], I. Perrault[1], S. Gerber[1], J.L. Dufier[2], A. Munnich[1], J. Kaplan[1], J.M. Rozet[1].* 1) Dept Genetics,INSERM U393, Hopital des Enfants Malades, Paris, France; 2) Ophthalmology, Hopital Necker, Paris , France.

The purpose of the present study was to understand the recurrence of Leber congenital amaurosis (LCA) in a child born to two patients affected with different retinal disorders: a typical GUCY2D-LCA phenotype for the mother and an early-onset severe retinitis pigmentosa (RP) for the father. The 18 coding exons of GUCY2D were screened for mutations by Denaturing High Pressure Liquid Chromatography (DHPLC) and direct sequencing using specific primers in the three affected individuals of this family. The screening of the GUCY2D gene revealed that the mother was compound heterozygous for two mutations: c.3043+4A>T and c.2943delG while the father carried a homozygous 4bp insertion in exon 19 (c.3236insACCA) leading to a 28 amino acids elongation of the protein. The affected infant was compound heterozygous for the paternal insertion and the maternal c.3043+4A>T mutation. Patients carrying mutations in the retinal guanylate cyclase (GUCY2D) gene were reported to be constantly affected with a particular form of Leber congenital amaurosis (LCA) defined as a "congenital stationary cone-rod dystrophy with high hypermetropia, panretinal degeneration and highly reduced visual acuity". We report here, the occurrence of an early-onset severe RP, different from LCA, resulting from homozygosity for a 4 bp insertion in GUCY2D. Interestingly, this mutation is excepted to result in a 28 amino acid elongation of the protein contrary to all GUCY2D mutations accounting for LCA which are expected to be null alleles. This report gives support to the existence of exceptional GUCY2D mutations accounting for a milder and different phenotype compared to the typical GUCY2D congenital stationary cone-rod dystrophy.

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Mapping of a Novel X-Linked Mental Retardation Syndrome to Xp11-Xp22. *Y. Wang, J.E. Martinez, F. Tan, C. Tuck-Muller, W. Wertelecki, T.-J. Chen.* Medical Genetics, Univeristy of South Alabama, Mobile, AL.

X-linked mental retardation is the most common and highly heterogeneous genetic condition including over 120 syndromic and nonsyndromic disorders. We reported mental retardation in a family with six affected males and two obligate carrier females in three generations. The first generation female carrier produced, from four different partners, four affected sons and a normal daughter. Her daughter went on to have two affected sons and two normal female offspring, all from different fathers, strongly indicating an X-linked pattern of inheritance. The affected males have moderate mental retardation, early global developmental delay, microcephaly and craniofacial dysmorphism, short stature, ptosis, muscle atrophy, joint contractures, pigmentary abnormalities and hypogonadism. Cytogenetic studies of the male patients were negative, and Fragile X syndrome was ruled out by DNA analysis of FMR1 gene. Thus, clinical manifestations in this family presented a previously unreported, X-linked mental retardation syndrome. Forty-eight fluorescent dye-labeled STR markers were used to determine the haplotypes of the family members. These markers covered entire X-chromosome with approximately 5cM resolution. Linkage analysis by the two point parametric method indicated the markers in the region of Xp11-Xp22 co-segregated with this disease phenotype. The maximal LOD score was obtained between markers DXS987 and DXS1068, a region containing the ARX and RSK2 genes. This region also overlaps with several other XLMR loci, such as MRX43, MRX54, and MRX59. Moreover, all patients in the second generation inherited the same X chromosome from the carrier of the first generation, and the female carrier in the second generation transmitted the X chromosome to all patients in the third generation, suggesting a strong segregation bias or meiotic drive. Further fine mapping and candidate gene screening is on going.

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Fragile X tremor/ataxia syndrome in female premutation carriers. *S. Russo[1], F. Cogliati[1], A. Gessi[1], S. Goldwurm[4], G. Neri[2], G. Scarano[3], R. Cilia[4], A.P. Verri[5], G. Pezzoli[4], L. Larizza[1,6].* 1) Ist.Auxologico Italiano,Milano, Italy; 2) Ist.Genetica Medica,Università Cattolica,Roma,Italy; 3) Osp.G.Rummo,Benevento,Italy; 4) CentroParkinson,I.C.P.,Milano,Italy; 5) Ist.Neurologico Casimiro Mondino, Pavia,Italy; 6) Genetica Medica,Università di Milano,Italy.

Fragile X tremor/ataxia syndrome,FXTAS,is a neurodegenerative syndrome arising,after the fifth decade,among 30% of male carriers of FMR1 gene premutation.FXTAS is defined by a)progressive intentional tremor and/or ataxia,associated with peripheral neuropathy,mild parkinsonism,autonomic dysfunction,cognitive deficits including loss of memory and executive functions,b)symmetric hyperintensities on T2 weighted MR images of the middle cerebellar peduncles and c)intranuclear inclusions in neurons and astrocytes throughout the brain in postmortem samples from adult male PM carriers.Among FMR1 PM female carriers FXTAS is rarer,being reported so far only 7 women with FXTAS,all displaying a milder clinical presentation than the FRAXTAS males,a finding discussed in the context of X inactivation.We report on 3 PM female carriers with a diagnosis of probable FX/TAS,identified within a sample of 72 FRAXA families.They were representative of a heterogenous clinical spectrum including:a)ataxia,MR anomalies and occasional tremor started at 55 y in a 115 CGG PM carrier,b)rest tremor,motor impairment at left hand, gait impairment started at 50 y with a few cerebellar traits and MR evidencing a mild cortical cerebellar atrophy in a carrier of 96 CGG and c)mild head tremor and intentional tremor of the left hand started at 61 y old in a 79 CGG carrier,whose detailed neurological evaluation is under study.The Activation Ratio to evaluate random X inactivation was assessed and found consistent with a skewed inactivation in all index cases.Examination of the healthy premutated sister of the first affected female evidenced random X inactivation versus a complete skewed inactivation in the patient.Our molecular and clinical data support the hypothesis of the protective role for random X inactivation in FRAXA premutated FX/TAS females and suggest a female carrier neurological phenotype distinct from the classification built on PM FRAXTAS males.

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Integrated Web-based Informatics for Array CGH. *C. Shaw, J. Fisk, A. Young, N. Whitehouse, S. Cheung, A. Patel, C. Chinault, P. Stankiewicz, J. Lupski, A. Beaudet.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Microarray CGH (a-CGH) is a powerful technique for determining variation in DNA copy number within clinical samples. The technique requires extensive sample tracking to ensure quality, and the data obtained are both massive in quantity and complex in content. We have developed a web based software to track, store, and analyze a-CGH results. As the collection of patients grows, the database also grows and becomes an ever more useful tool for clinical decisions. The database also serves as a resource to understand variation in copy number. The software and database are particularly useful to discriminate benign polymorphisms from clinically relevant alterations.

Our system provides a myriad of views to the array data. The first view is a single patient report which provides a comprehensive and orderly presentation of the experimental outcome for a single case. The patient report shows the raw, normalized, and integrated data on all clones as well as quality information on the DNA sample. This display provides variance information and the test-statistic to determine outcomes for individual clones and regions.

The system also provides dynamic queries for individual clones and genomic regions whereby all data available within the database - across all a-CGH results -- can be considered against the values observed in any particular case. These reports provide a powerful tool for distinguishing benign variation from those implicated in disease.

Our system is web based and therefore highly distributed. We utilize sophisticated backend analysis performed in the R open source statistical computing environment, and we use the MySQL database engine. As our patient database and array platform expand we believe this software will continue to be important. More generally, the web-based information system provides a model for translational research whereby molecular data from new methods are directly relevant to clinical practice.

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Chromosome 15q abnormalities in autism: Phenotypic studies and molecular genetic analyses in 15q11-q13 and 15q23. *M. Smith, P.L. Flodman, M.E. Bocian, K. Osann, C. Mohdahl, B. Moore, P.A. Filipek, M.A. Spence.* Dept Pediatrics, Univ California, Irvine, Irvine, CA.

We report results of clinical, psychological and molecular genetic studies on five patients with autism who have abnormalities of chromosome 15q.Testing including autism evaluation (ADOS, ADI or CARS), IQ testing (Stanford Binet, Mullen or WISC) and language assessment. The Vineland Adaptive behavior test was carried out. FISH studies and microsatellite polymorphism analyses revealed that four patients have duplications of proximal 15q. One patient has a maternally derived interstitial duplication of SNURF-SNRPN and UBE3A loci; the GABA receptor loci are not duplicated. His phenotype differs from that in two other patients who have maternally derived 15q inverted duplications that extend from the centromere to BP4 and include the GABA receptor loci. The phenotypic differences suggest that verbal and social skills are negatively impacted by increased dosage of SNURF-SNRPN and UBE3A, while extra-copies of the GABA receptor gene cluster significantly impact performance IQ and adaptive behavior. We describe a patient with mild autism who has a paternally derived duplication of the PWS-AS gene region. It is not clear why only a subset of patients with paternally derived duplications of this region manifest symptoms. It is possible that one factor is the co-occurrence of mutations in the imprint control region. Studies on a fifth autism patient revealed SIN3A hemizygosity. SIN3A, histone deacetylases and MECP2 function as transcription co-repressors. The MECP2 complex to binds to methylated DNA in the SNURF-SNRPN region, and impacts imprinting and expression of UBE3A. MECP2 is deficient in Rett syndrome. Taken together results in these patients reveal a possible common pathway in the pathogenesis of some forms of autism e.g. altered expression of imprinted genes on 15q. Occurrence, in a patient with autism, of a deletion in 15q22-q23 that extends from 70, 000 to 73,600KB is of further interest in light of recent linkage studies that revealed that in female-male and male-female autistic sib-pairs maximal evidence was found with markers that map between 60,000 and 72,000 KB.

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Genomwide comparison of human and mouse Common Fragile Sites and molecular characterization of five mouse Common Fragile Sites that are syntenic to human Common Fragile Sites. *E. Schrock[1, 2], K. Stout-Weider[1, 2], A. Matthaei[1, 2], K. Hermann[3], T. Heiden[1, 2], A. Helmrich[1, 2].* 1) Institute of Clinical Genetics, University of Technology, Dresden, Germany; 2) Institute of Medical Genetics, Charité Universitätsmedizin Berlin, Germany; 3) Signature Diagnostics, Potsdam, Germany.

Common fragile sites (CFSs) are expressed as chromosome gaps in cells of different species including human and mouse as a result of the inhibition of DNA-replication. They may serve as hot spots for DNA-breakage in processes such as tumorigenesis and chromosome evolution. This study presents a comprehensive analysis using conventional and molecular cytogenetic mapping of CFS frequencies in two mouse strains, BALB/c and C57BL/6, and in human probands. We show that CFS expression levels in chromosome bands are conserved between the two mouse strains and between syntenic mouse and human DNA segments. Furthermore, five additional mouse CFSs were found to be homologous to human CFSs on the molecular cytogenetic level (Fra2D-FRA2G, Fra4C2-FRA9E, Fra6A3.1-FRA7G, Fra6B1-FRA7H and Fra12C1-FRA7K) increasing the number of syntenic CFSs to eight. Focussing on human chromosome 7 we compared frequencies of malignancy and non-malignancy associated chromosome rearrangements to the expression pattern of CFSs. Using array-CGH-analysis and multicolor-FISH-mapping we located two amplification breakpoints of the breast cancer cell line SKBR3 within the CFSs FRA7G and FRA7H. Additionally, in contrast to previous reports, DNA helix flexibility is not increased in all so far molecularly defined 13 human and eight mouse CFSs compared to large non-fragile control regions.

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CHK1 regulates fragile site stability. *S.G. Durkin, T.W. Glover.* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Common fragile sites are site specific gaps and breaks seen on metaphase chromosomes when DNA synthesis is partially inhibited. These conserved regions are often rearranged in cancer cells, and may serve as signatures of replication stress during early stages of tumorigenesis. We have previously shown that the ATR pathway is central in maintaining chromosomal integrity at these regions, most likely by responding to stalled or delayed replication forks. Here we have characterized the roles of the CHK1 and CHK2 kinases, targets of ATR which are phosphorylated and activated in the DNA damage checkpoint. Using RNAi, we depleted cellular CHK1 and CHK2 pools and examined the effects on total chromosomal stability and on the expression of the two most common fragile sites, FRA3B and FRA16D. In addition, we examined the effects of the low concentrations of aphidicolin (APH) used to induce fragile site breakage, but not inhibit replication entirely, on the post-translational modifications of both CHK1 and CHK2 and found ATM-independent phosphorylation of both proteins following treatment with low dose APH. We observed two phenotypes in cells depleted for CHK1 that were not observed in cells depleted for CHK2. Approximately 10-40% of these cells displayed discrete numbers of gaps and breaks, largely at specific common fragile sites, and ~60-90% of cells displayed extensive chromosome breaks and fragmentation exceeding scoreable levels. To determine if this chromosomal fragmentation was a result of apoptosis, CHK1 depleted cells were analyzed by annexin V staining for phosphatidylserine residues exposed on the outer cytoplasmic cell membrane, an early apoptotic event, and by treating CHK1 depleted cells with the apoptosis inhibitor zVAD analyzing chromosome breakage by both TUNEL staining and metaphase chromosome analysis. These analyses showed that the chromosome fragmentation resulting from CHK1 deficiency was not a result of apoptosis, but rather appeared to result from a requirement for CHK1 during normal replication. Our results demonstrate CHK1 is essential in regulating the integrity of fragile site regions, likely a result of its G2/M checkpoint function, and further delineate the importance for CHK1 in regulating S phase events.

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Aplasia Cutis Congenita in an infant with de novo t(1;20)(p34.1;q13.1). *S. Gupta[1], L. Mehta[2], A. Shanmugham[2], A. Thomas[1], P. Koduru[1].* 1) Laboratory Medicine, North Shore University Hospital, Manhasset, NY; 2) Medical Genetics, Schneider's Childern's Hospital at NSUH, NY.

Aplasia cutis congenita (ACC) is a rare disorder characterized by localized absence of all skin layers. The defect may also involve deeper tissues such as muscle and bone, extending to the dura matter when the scalp is involved. The defect may be isolated or may occur in association with malformation syndromes such as Adams-Oliver syndrome or chromosome abnormalities and with other mechanisms such as twinning, trauma, specific teratogens or intrauterine infections. Chromosomal abnormalities have been rarely reported in association with ACC. We present a newborn with a de novo t(1;20) and cutis aplasia of the scalp. Amniocentesis was performed on a 34 year old, G5P2 woman at 15.6 weeks of gestation for reasons of maternal age and history of spontaneous abortions. The mother took Synthroid and Paxil during the pregnancy. Chromosome analysis of cultured amniocytes revealed a balanced translocation (1;20)(p34.1;q13.1). Both parents had normal karyotypes. Fetal echocardiogram and comprehensive sonogram were normal. The baby was born full term and noted to have a single rounded area of cutis aplasia (1 cm x 1 cm), without a bony defect, on the central occipital region. No other dysmorphic features or birth defects were present. Blood chromosomes confirmed the balanced translocation. Khan et al (1995) reported ACC in association with an unbalanced karyotype, monosomic for 12q and trisomic for 1q; another patient with de novo del(15)(q15.2q21.2) had pronounced scalp defects, dysmorphic features and mental retardation (Koivisto et al 1999). Structural rearrangements of chromosomes, including apparently balanced translocations, may result in subtle loss or gain of genomic material or influence gene function due to position effect and may indicate candidate regions of the genome to explore. In cases where there is an absence of another etiology for ACC, chromosome analysis should be performed. In our patient, further molecular studies are indicated to rule out submicroscopic deletions in the presence of a balanced translocation.

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MOLECULAR CHARACTERIZATION OF AN INVERTED DUPLICATION OF CHROMOSOME X WITH TERMINAL Xp DELETION. *C. DUPONT[1], A. LEBBAR[1], C. TEINTURIER[2], F. BAVEREL[1], G. VIOT[3], D. LETESSIER[1], P. AUBOURG[2], J.M. DUPONT[1].* 1) Service dHistologie Embryologie Cytogénétique, Hôpital Cochin, Paris; 2) Service de Neurologie et endocrinologie Pédiatrique, Hôpital Saint- Vincent de Paul, Paris; 3) Génétique Médicale, Maternité Port Royal, Hôpital Cochin, Paris.

We report on a 6-year-old boy [GL] referred for karyotyping and searching for SHOX microdélétion. The most significant clinical findings in this boy were small stature, Madelung deformity, facial dysmorphy, developmental milestones moderately delayed and deficits in attention and memory abilities. Classical cytogenetic analysis had not shown any rearrangement. Fluorescence in situ hybridization (FISH) investigations using cosmidic probe M34F5 covering SHOX gene (Xp22.33) showed a microdélétion of this region. Given that GL phenotype includes clinical findings not classical in Leri-Weill syndrome, we tested subtelomeric Xp probe (CTC-839D20, Xp22.33) and found it deleted too. Interestingly, characterization of deletion proximal border using commercial probes localized in Xp22.3 (STS and KAL1), showed a partial duplication of Xp associated to the deletion. Then with BACs help, we finally diagnosed an inverted duplication Xp22.31-Xp22.32 (13,7 Mb) included STS, VCX-A and KAL1 genes associated to a terminal Xp deletion Xp22.33-Xpter (3,6 Mb) included SHOX and ARSE genes. Inverted duplications with a terminal deletion are complex chromosome rearrangements well defined on 8p and some other chromosomes (2q, 3p, 4p, 9p, 11p). But only one case has been described on chromosome X (Milunsky et al 1999). Interchromosomal or interchromatid mispairing leading to nonallelic homologous recombination appeared to originate this rearrangement. Short arm of chromosome X is a rich-genes region that we can discuss the phenotype of this child. Indeed, he has a mixed phenotype create by the loss of Xp subtelomeric region (attention and memory deficit), of SHOX gene (Leri-Weill syndrom) and ARSE gene (Chrondrodysplasia Punctata) and create by a chromosome X partial functional disomy including some important genes in human pathology (STS, VCX-A and KAL1).

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FISH analysis to differentiate Type I and Type II deletions in Prader-Willi and Angelman syndrome patients.
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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two distinct syndromes that can result from deletion of 15q11.2-q12, uniparental disomy, or imprinting defects. Deletions of the 15q11.2-q12 region are recurrent and mediated by non-allelic homologous recombination events at low-copy repeats (LCRs) in this region. Type 1 deletions are larger deletions spanning breakpoint BP1 and the more distal BP3 breakpoint, and the smaller (by about 500kb) Type II deletions span BP2 and BP3. Depending on the extent of the deletion, behavioral differences have been described in both PW and AS patients. In both PWS and AS, Type I patients have a more severe phenotype including more severely delayed development and autistic features. Distinguishing between Type I and Type II deletions is useful in counseling PWS or AS patients, but current methods are research-based molecular assays. We have developed a home brew probe from DNA clones located between BP1 and BP2. Using fluorescent in situ hybridization (FISH) techniques, these probes can be used to distinguish between Type I and Type II deletions. Testing was performed on a series of 35 patients exhibiting a FISH deletion of SNRPN or D15S10. Six of 19 (32%) PWS and four of ten (40%) AS cases exhibited a BP1 deletion, as did 4 of 6 (67%) patients referred with an unspecified diagnosis. Ten patients with a supernumerary dic(15) also were tested; in seven cases, the probe was present on both arms of the dic(15), in two cases the probe was absent from both arms of the dup(15), and one case exhibited the BP1-BP2 probe without SNRPN on both arms of the dic(15). In each of these cases, the normal 15 homolog had a normal FISH pattern. To further investigate the possibility of BP1 deletions in patients with autistic-like features or attention deficit disorder, twenty patients were tested but none showed a BP1 deletion. We believe the use of this probe as a reflex test in samples with a deletion of SNRPN or D15S10 provides a sensitive technique to identify BP1 deletions in patients with PWS or AS.

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T Lymphocytes from Older Individuals with Down Syndrome and Dementia Exhibit Telomere Shortening. *E.C. Jenkins[1], M.T. Velinov[1], L. Ye[1], H. Gu[1], E.C. Jenkins Jr.[1], D. Pang[1], D.A. Devenny[1], W.B. Zigman[1], N. Schupf[1, 2], W.P. Silverman[1].* 1) New York State Institute for Basic Research in Developmental Disabilities, Staten Island; 2) Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York.

We have previously reported preliminary findings that telomeres, chromosome ends consisting of highly conserved TTAGGG repeats, were shorter in metaphase and interphase preparations from short-term whole blood cultures of women (49-59)with Down Syndrome and dementia (n=6) compared to age-matched women with DS without dementia (p=.028)(ASHG, 2004). Using quantitative telomere protein nucleic acid FISH analysis, we have now confirmed and extended our observations in eleven adults with DS (10 females and one male, a total of 22 individuals, 49-63 years), who were age- and sex-matched to adults with DS and no dementia. Subjects were ascertained through the NYS Developmental Disability Service System and followed at 18 month intervals for 3 cycles of data collection. Subjects were classified as demented if they developed progressive memory loss, disorientation, functional decline over a period of at least one year, and had a clinical diagnosis of AD. Cells in metaphase and interphase from demented versus non-demented subjects consistently exhibited increased telomere shortening as a function of reduced light intensity in cells from demented subjects. Our first study of a male with trisomy 21 and dementia suggests that our findings are not limited to females. Our first study of a female with trisomy 21 and a dementia status of questionable (indicating substantial uncertainty regarding dementia status, with some indications of mild functional and cognitive declines) compared to a female with trisomy 21 who was classified as non-demented, exhibited increased telomere shortening in T lymphocytes from the individual with a dementia status of questionable, thus strengthening our hypothesis that telomere shortening is a candidate as a biomarker of dementia status. This work was supported in part by NYS OMRDD, Alzh. Assoc. grants IIRG-99-1598,IIRG-96-077; by NIH grants PO1 HD35897, RO1 HD37425, RO1 AG014673, and RO1 AG14771.

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Incidence of Microduplication 22q11.2 in Patients Referred for FISH Testing for Velo-Cardio-Facial and DiGeorge Syndromes. *P.D. Cotter[1,2], H. Nguyen[1], G. Tung[1], K.A. Rauen[2,3].* 1) Department of Pathology, Childen's Hospital Oakland, Oakland, CA; 2) Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA; 3) Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA.

Microduplication of 22q11.2 has recently emerged as a new chromosomal syndrome. Previous reports ascertained microduplication 22q11.2 patients among referrals for FISH testing for velo-cardio-facial and DiGeorge syndromes (VCF/DGS). In one large series, 2% of patients had the microduplication. To further evaluate the incidence of the 22q11.2 microduplication, we prospectively screened all VCF/DGS patient referrals over a two year period from April 2003 through May 2005. In total, 372 patients were referred for FISH testing with VCF/DGS as part of the clinical differential diagnosis. FISH analysis was performed using either the TUPLE1/ARSA probe set or the D22S75/n85a3 probe set according to the manufacturers instructions. Twenty metaphases were scored for the microdeletion by FISH in each patient. In addition, 50 interphase nuclei were scored to look for the additional signal associated with the 22q11.2 microduplication. In a control sample, both the TUPLE1 and D22S75 probes readily identified the microduplication at interphase, but somewhat less reliably at metaphase. We identified 30 patients (8%) with 22q11.2 deletions by FISH on metaphase chromosomes from the 372 patients referred. No patients were identified with the 22q11.2 microduplication by interphase FISH. Our data suggest that in an unselected population of patients referred for VCF/DGS testing the incidence of the microduplication might be lower than previously reported. Although the incidence of microduplication 22q11.2 might be low in the VCF/DGS population undergoing FISH testing, additional screening of interphase nuclei to look for the duplication would seem reasonable. A systematic evaluation of more diverse patient population, including normal individuals will better characterize the frequency and phenotype for microduplication 22q11.2 syndrome and define a more appropriate target population for testing.

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Complete trisomy 17p syndrome in a girl with der(14)t(14;17)(p11.2;p11.2). *F.M. Mikhail, D. McIvried, R.L. Holt, L. Messiaen, M. Descartes, A.J. Carroll.* Department of Genetics, University of Alabama at Birmingham, AL.

Complete trisomy of the short arm of chromosome 17 has been previously described, but is quite rare. It has been suggested to comprise a new syndrome owing to the similarities in phenotype. The phenotypic findings include, pre- and post-natal growth retardation, mental and motor retardation, skeletal anomalies, clinodactyly of fingers, short neck and peculiar facial abnormalities including, microcephaly, down slanting palpebral fissures, hypertelorism, poorly shaped ears, smooth philtrum, micrognathia and high-arched palate. Here we report a seven year old girl with multiple congenital abnormalities and dysmorphic features. She was first studied cytogenetically immediately after birth and was reported to have a 46,XX,add(14p) karyotype. Parental chromosomes were normal. At age 7 the patient was re-evaluated for history of chromosome abnormality. High resolution banding analysis demonstrated that the extra material on 14p was suggestive of 17p. FISH confirmed this with both the Smith-Magenis region probe (17p11.2) as well as the 17p subtelomeric probe seen to hybridize to the p arm of der(14) indicating near complete 17p trisomy. Also, a chromosome 17 centromeric probe did not hybridize to the der(14) suggesting that the breakpoints were p11.2 for both chromosomes 14 and 17. The final karyotype of this patient was 46,XX,der(14)t(14;17)(p11.2;p11.2)de novo. On physical examination she displayed remarkable similarities with the previously reported complete trisomy 17p phenotype. These included, growth and mental retardation, decreased reflexes with a mixture of hypotonia and hypertonia, microcephaly, receding forehead, low anterior and posterior hairlines, flared eyebrows, slanting palpebral fissures, cupped ears, smooth philtrum, high-arched palate, triangular and coarse facies, short neck, scoliosis, 5th finger clinodactyly and no speech. She also had a history of bilateral cataract as well as club feet. Our findings strongly support the distinctive phenotype of a Complete trisomy 17p syndrome. We are currently conducting molecular studies using microsatellite markers to determine the parental origin of the extra 17p as well as recombination events.

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Microdeletion and Microduplication of Chromosome 22q11.2: Four-Years Experience. *J. Lee[1], N. Sebastian[2], S. Hassed[1], R. Seely[1], J. Mulvihill[1], S. Li[1].* 1) Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Oklahoma School of Science and Mathematics, Oklahoma City, OK.

Microdeletion of chromosome 22q11.2 is responsible for a broad spectrum of developmental disorders including DiGeorge syndrome, velocardiofacial syndrome, and conotruncal cardiac defects. Although a microduplication of 22q11.2 has many manifestations, these patients have distinctive facial anomalies including widely spaced eyes, down-slanting palpebral fissures, and superiorly placed eyebrows. Since 2000, our laboratory has provided fluorescence in situ hybridization (FISH) testing for microdeletion/microduplication of chromosome 22q using a commercial DNA probe, TUPLE1. 259 consecutive patients with clinical features suggestive of a microdeletion 22q11.2 have been referred by clinical geneticists, cardiologists, or pediatricians. Thirty-five patients had a microdeletion of the TUPLE1 gene; three index patients had microduplication of TUPLE1. Two patients with the microdeletion also had a loss or gain of other chromosomal material due to unbalanced translocations involving chromosome 22. As expected, variable clinical features were found in the patients with the microdeletion or microduplication of chromosome 22.

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Microduplication 22q11.2 syndrome : a cause of cognitive problems - report of 2 families. *W. Courtens[1], W. Wuyts[1], A. Laridon[2], I. Schramme[3], R. van Luijk[1], S. Scheers[1], N. Peeters[1], J. Wauters[1].* 1) Department of Medical Genetics, University Hospital Antwerp, , Antwerp, Belgium; 2) Department of Child Neurology, University Hospital Antwerp, Antwerp, Belgium; 3) Centre for Developmental Disorders Antwerp, Antwerp, Belgium.

We report on two unrelated families where the probands presented with psychomotor retardation. In the first family the proband was an 8-year-old boy referred because of psychomotor retardation, behavioural problems and mild facial dysmorphism, in whom a microduplication 22q11 was discovered. The standard karyotype of the patient was normal 46,XY but FISH analyses 22q11.2 showed the presence of three signals for TUPLE1 probe in all interphase nuclei. Complementary investigations showed that the patient had, in addition to his developmental delay and behavioural problems, asymmetric perceptive hearing loss and a small right kidney. His father and one brother, also presenting with mental retardation and behavioural anomalies, had the same microduplication. In the second family only the proband presented with mild psychomotor retardation. The same microduplication was discoved in her asymptomatic mother. Further FISH studies allowed to identify a duplication between 2.1 and 3.4 Mb in both families. Microduplications of the 22q11.2 region have only recently been observed examining interphase cells by FISH with TUPLE1 in patients referred for DG/VCFS by Ensenauer et al. [2003] who found a duplication in 1.5% of unrelated patients. This finding has been verified in a prospective study on 200 cases referred for DG/VCFS, in which one patient (0.5%) with three copies of TUPLE1 was found. Since then only a few patients with microduplication 22q11.2 were described. We compare the clinical findings of our cases with those of other published cases in an attempt to further identify common phenotypic aspects.

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Analysis of achiasmate bivalents in pachytene cells from 8 normal men. *F. Sun[1,2], M. Oliver-Bonet[1,2], T. Liehr[3], H. Starke[3], P. Turek[4], E. Ko[2], A. Rademaker[5], R.H. Martin[1,2].* 1) Medical Genetics, University of Calgary, Calgary, Canada; 2) Genetics, Alberta Children's Hospital, Calgary, Canada; 3) Institute of Human Genetics and Anthropology, Jena, Germany; 4) Urology, Obstetrics and Gynecology and Reproductive Sciences, University of California, San Francisco; 5) Northwestern University, Chicago, USA.

Meiotic exchange, or recombination, is required to ensure the proper disjunction of homologous chromosomes to opposite poles at the first meiotic division. Bivalents with no recombination foci (future achiasmates) are unable to orient properly on the metaphase plate or to segregate chromosomes to daughter cells. Achiasmate bivalents are known to lead to meiotic arrest in various organisms. We identified individual nonexchange bivalents in pachytene cells from testicular biopsies of 8 normal human males (3 cancer patients with normal spermatogenesis and 5 vasectomy reversals). An immunocytogenetic approach allowed analysis of pachytene cells by using antibodies to detect the synaptonemal complex (SC),centromere and sites of recombination (MLH1).698 pachytene cells and 16,054 individual bivalents were identified with centromere-specific multicolour FISH. A total of 26 achiasmates (SCs with 0 MLH1 foci) were found and of these chromosomes 21 (34.6%) had a significantly higher proportion than chromosome 10 (0%), 12 (3.8%), 13 (0%), 15 (7.7%), 16 (3.8%), 17 (7.7%), 18 (3.8%) and 20 (3.8%) ($P<0.05$, Fishers exact test). Chromosome 14 (11.5%) and 22 (23%) also had lower frequencies of achiasmate bivalents but this did not reach statistical significances. This suggests that chromosome 21 is most susceptible to having no recombination foci and thus would be more susceptible to nondisjuction during spermatogenesis. This is consistent with previous observations from sperm karyotyping and FISH analysis which demonstrate that chromosomes 21 has a significantly increased frequency of aneuploidy compared to other autosomes. Chromosome 21 has the lowest mean frequency of recombination sites of all the autosomes (1.000.23) and thus it is most susceptible to having an achiasmate bivalent which can subsequently result in a sperm aneuploidy for chromosome 21.

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Cytogenetic Approaches to Identifying Genes for Hearing Impairments: Gene Discovery in an inv(7)(q21.3q35) and an inv(5)(q15q33.2). *K. Kocher[1], C.C. Morton[1,2].* 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA.

Hearing loss is a common sensory disorder with an estimated incidence of 1 in 1000 human births. Approximately half of these cases are attributed to environmental factors, while half are due to genetic causes. Genes with a role in the auditory system have been identified through both genetic linkage studies of families with heritable deafness and positional candidate gene approaches. Another method for gene discovery is to ascertain deaf individuals that carry balanced chromosomal rearrangements and identify disrupted or disregulated genes at the site of rearrangement. Here, FISH experiments were performed to map the breakpoint regions of a family with moderate hearing loss and of an individual with profound deafness. The family with moderate hearing loss consists of five affected members spanning three generations who carry a balanced paracentric inversion, inv(7)(q21.3q35). Successive FISH experiments were performed to map the 7q21.3 breakpoint within BAC RP11-879e11 and the 7q35 breakpoint within BAC RP11-643a21. Further analysis showed that the 7q21.3 breakpoint lies approximately 67 kb and 80 kb centromeric of the *DLX6* and *DLX5* genes, respectively. *DLX5/6* are important for proper development of the middle and inner ear and disregulation of these genes are likely to cause hearing loss in this family. The individual with profound deafness carries a *de novo* paracentric inversion, inv(5)(q15q33.2). FISH mapping has narrowed the 5q15 breakpoint to approximately 300 kb and the 5q33.2 breakpoint to approximately 500 kb. *NR2F1*, a gene highly expressed in human fetal cochlea, lies within the 5q15 breakpoint region and may contribute to the etiology of hearing loss in this individual.

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A girl with an apparently balanced chromosome inversion (5)(p13q13) and atypical autism and mental retardation. *Z. Chen[1], H.Y. Li[1], X.H. Li[2], C.N. Huang[1], X.M. Son[1], J. Xu[3].* 1) Medical Genetics; 2) Neurology, First Affiliated Hospital, Zhongshan Medical College, Sun Yat-sen University, Guangzhou, PR China; 3) Cytogenetics, London Health Sciences Centre and University of Western Ontario, Canada.

Our recent reviews (Xu et al, Current Genomics, 2004, 5(4):347-364) showed that chromosome anomalies were found in ~7.4% (129/1749) of autism patients. There is an increased rate of apparently balanced chromosomal rearrangements (ABCR) in autism and 65% (17/26) of the breakpoints of ABCR have cryptic chromosomal rearrangements (CCR) of potential significance. This finding indicates that positional cloning of breakpoints is a very promising strategy in autism. We present a case of chromosome 5 inversion associated with atypical autism and mental retardation (MR). The female patient was born following an uncomplicated pregnancy at 39 weeks gestation with a birth weight of 2750g. She first walked at age 14 months and was found with moderate MR (IQ 48) and abnormal functioning in social interaction at age 2 years. She was diagnosed with atypical autism at age 6 years. She is shy, plays by herself, and lacks initiatives to share enjoyment, interests, achievement and reciprocity with other people. She has no speech and language development. Her vision and hearing are normal. The magnetic resonance imaging of her brain showed no anomalies. The family history is uneventful with regard to neurodevelopmental conditions. Routine cytogenetics (at ~550 G-band level) of the peripheral blood revealed a female karyotype with an apparently balanced and de novo pericentric inversion, 46,XX,inv(5)(p13q13). The breakpoints 5p13 and 5q13 have been previously reported to be implicated in 3 and 2 autism cases, respectively. This case waits for further molecular cytogenetics study of the breakpoints. More case reports and use of cytogenomics (e.g. microarrays) for detecting and screening for CCR in ABCR should help gene discovery in autism and/or MR.

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Subtelomeric familial translocation t(8;12)(p23.1;p13.1) leading to deletion 8pter-p23.1 and duplication 12pter-p13.1: molecular cytogenetic analysis and clinical phenotype in two siblings. *A. Blumenthal[1,2], D. Verbaan[1], J. Halbgewachs[1], E. Kolomietz[1,2].* 1) Laboratory Medicine and Genetic Services, Lakeridge Health Corporation, Oshawa, Ontario, Canada; 2) Department of Pathology and Lab. Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Unbalanced cryptic subtelomeric chromosomal rearrangements represent a significant cause of unexplained moderate to severe mental retardation. The use of subtelomeric FISH probes has greatly supplemented conventional chromosome analysis in detecting submicroscopic anomalies in patients with seemingly normal karyotypes. We report on two siblings of different sex and apparently normal karyotypes who had the same syndrome of minor anomalies: macrocephaly and global developmental delay. Neither have cardiac or renal abnormalities or any behavioural concerns at present. Family history revealed multiple first trimester miscarriages. Parental chromosomes studied in mid nineties appeared to be normal. These findings raised suspicions of a cryptic chromosome rearrangement. A translocation t(8;12)(p23.1;p13.1), balanced in the mother and the same unbalanced adjacent -1 segregation variant in the sibs with loss of terminal 8p and gain of terminal 12p regions, was verified by FISH using whole chromosome painting and subtelomeric probes. Some of the features observed in affected individuals are characteristic of known syndromes involving either 8p (upslanting palpebral fissures, short nose with a wide bridge and prominent colemma, thin upper lip, moderate mental retardation in 8pter deletion syndrome) or 12p (macrocephaly, large for gestational age, full cheeks in distal 12p trisomy). This familial case demonstrates the value of subtelomeric FISH in the diagnosis of submicroscopic chromosome abnormalities in those patents for whom routine chromosome analysis does not reveal result adequate to explain clinical findings.

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Emerging evidence for a novel microdeletion syndrome at 15q24 with autism/PDD. *C.Lese Martin[1], L.F. May[1], K. Muralidharan[1], B-M. Anderlid[2], J. Schoumans[2], D. Rita[3], M. Smith[4], D.H. Ledbetter[1].* 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Dept Molecular Medicine, Karolinska Institute, Stockholm, Sweden; 3) Lutheran General Hospital, Park Ridge, IL; 4) Dept Pediatrics, Univ California, Irvine, CA.

With the increasing use of molecular cytogenetics in the investigation of individuals with unexplained mental retardation or developmental delay, microdeletions and microduplications across the genome are being unveiled. We have evidence for a novel microdeletion syndrome involving the *PML* locus on chromosome 15 at band q24. To date, we have ascertained 5 cases with *de novo* deletions of this region that were referred for clinical testing by Fluorescence *In Situ* Hybridization (FISH) for telomere analysis or to rule out Prader-Willi (PWS) or Angelman syndromes (AS). All 5 cases were serendipitously detected: a clone corresponding to the *PML* locus, which is included as a control in a commercially available assay for telomere screening and PWS/AS, was deleted in all individuals. We have begun to characterize the size, genomic content and parental origin of this deletion. Three deletions have been mapped using a combination of FISH and array Comparative Genomic Hybridization using a custom chromosome 15 array with clone coverage at a density of ~600 kb in the region of interest. Two deletions were ~3 Mb in size and the third was ~9.4 Mb. This region includes multiple copies of duplicon sequences which may mediate these imbalances. In addition, there is preliminary evidence to indicate a parent of origin effect. Of the three cases studied, two are paternally inherited and one is maternally inherited. The individuals with paternally derived deletions are markedly fair, well below normal curves for height and weight and have autism. The patient with a maternally inherited deletion exhibits hyperpigmentation, has been noted to be obese and is in the 97th centile for height; he has pervasive developmental delay (PDD). Detailed clinical and molecular cytogenetic characterization of additional individuals with this deletion will add to the growing description of genomic imbalances that are delineating new clinical syndromes.

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A girl with 22q13.33 subtelomeric deletion and very friendly personality! *C. Prasad[1], C. Campbell[1,2], S. Conachar[1], J. Jung[1], J. Xu[3].* 1) Pediatrics and Genetics; 2) Neurology; 3) Cytogenetics, London Health Sciences Centre, University of Western Ontario, Canada.

Deletion of 22q13.33 or McPhelan-McDermidt syndrome is reported in more than 75 cases. The common clinical features of the syndrome include developmental delay (DD, in particular speech), hypotonia, relatively well preserved growth and pervasive developmental disorder. A 4 year old girl presented with severe DD, including significant speech delay (vocabulary of approximately 40 words). She had Dandy Walker variant. The parents are non consanguineous with no positive family history. The proposita had an extremely friendly personality and was giving hugs to strangers. Parents reported a high threshold to pain. There were no seizures. She had significant microcephaly with a head circumference of 45.8 cm (below 3rd centile); weight and height were on the 50th centile at 15.3 kg and 99.2 cm, respectively. Facial features revealed a narrow forehead, normal ears and no cleft palate. She had clinodactyly of 5th fingers bilaterally. The cardiovascular and abdominal examinations were normal. Neurological examination was significant for central hypotonia with a wide based ataxic gait. Numerous investigations including metabolic work up were normal. Cytogenetics of the peripheral blood showed a normal female karyotype (~650 band level). FISH for Angelman/Prader-Willi region at 15q11-13 detected no anomalies. FISH screening using a set of 41 subtelomeric probes identified a deletion of the probe at 22q13.33. This deletion is confirmed by FISH using probe N85A3 at 22q13.3. It is de novo deletion since the parents had a normal karyotype by G-banding and the telomere FISH. The microcephaly and Dandy Walker variant along with an extremely friendly personality are strikingly unusual findings for this syndrome, which is usually associated with autistic features and poor social interactions. Overlapping findings with Angelman syndrome are previously described by Precht et al 1998. This case emphasizes significance of telomere FISH screening and other cytogenomics studies in patients with idiopathic mental retardation and other clinical findings.

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Mosaic Variegated Aneuploidy (MVA) with intact spindle checkpoint. *V. Jobanputra[1], J. Rockey[1], M. O'Driscoll[2], P. Jeggo[2], D. Warburton[1].* 1) Columbia Univ, New York, NY; 2) Univ of Sussex, East Sussex,UK.

MVA is characterized by growth retardation, severe microcephaly and constitutional mosaicism for aneuploidy involving multiple chromosomes. A defective mitotic-spindle checkpoint was demonstrated in 2 cases after treatment of cells with colcemid (Matsuura et al. AJHG, 2000). Recently mutations of both BUB1B alleles have been described in 5 families with MVA. Each case carried 1 missense mutation and 1 mutation that resulted in protein truncation or absent transcript (Hanks et al, Nat Genet, 2004). However, it is not known what proportion of MVA is due to BUB1B mutation.

In 1991 we described a 17-year-old girl with MVA who had clinical features resembling Seckel syndrome (Warburton, Ann Genet, 1991). We recently tested the mitotic spindle checkpoint in a fibroblast (GM09814) and a lymphoblastoid (GM09703) cell line from our patient. Both lines accumulated mitotic cells normally after treatment with nocadozole, indicating an intact spindle checkpoint. Direct sequencing of the entire coding region also did not reveal any mutations in BUB1B. Since GM09703 was designated as Seckel syndrome in the Coriell database, it was also analyzed by Alderton et al. (HMG, 2004) in a study of the ATR-dependent DNA damage response in Seckel patients. Previously a synonymous hypomorphic mutation of ATR, a gene in the repair pathway that responds to replication stalling, was found in two related Seckel syndrome families (O'Driscoll et al. Nat Genet, 2003). Our MVA cells exhibit many of the same features as the ATR-mutant cells, including reduced UV-induced H2AX formation, defective UV-induced G2/M arrest, and increased levels of nuclear fragmentation and micronuclei formation after treatment with agents that induce replication stalling. Chromosome breakage was not increased by aphidocolin treatment. However, no mutations were found in ATR and its binding partner ATRIP, or in H2AX, RPA1, RPA2 and RPA3.

Our data show that MVA can arise through mutations other than in BUB1B, and may involve errors in the G2/M checkpoint rather than the spindle checkpoint.

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Marker chromosomes: New insights and worries. *L.F. May, C. Works, C. Lese Martin, D.H. Ledbetter.* Dept Human Genetics, Emory Univ, Atlanta, GA.

Common assumptions regarding nonacrocentric supernumerary marker chromosomes (SMC) include 1) they arise by breaks in the p and q arms, and could contain genes from one or both arms; 2) phenotypic severity is positively correlated with percentage mosaicism; and 3) that SMCs are found in association with two structurally normal homologs. We present a family and review the literature on marker chromosomes associated with pericentromeric deletions which challenge these assumptions. Prenatal analysis for an elevated serum screen identified a fetus mosaic (33%) for a SMC identified as chromosome 4. The same mar(4) was identified in the mother (66% of cells) who had unilateral ear deformities and blurred vision. FISH studies with clones for proximal 4p and 4q showed the childs marker to be positive only for BAC clones from the p arm, resulting in partial trisomy for a 5 Mb segment. FISH studies of the mother showed the same hybridization results for the mar(4), but revealed a deletion of these probes on one of her chromosome 4 homologs, indicating a balanced carrier state for a cryptic pericentromeric deletion corresponding to the mar(4). Ironically, in the presence of a deleted homolog, clinical severity will be *inversely* related to the percentage of cells with the extra marker chromosome present. Review of the literature revealed eleven similar cases of interstitial deletions involving centromeric alpha satellite associated with the formation of a SMC. Although this mechanism has been considered rare, other lines of evidence suggest that a majority of marker cases are positive for unique sequences from only one arm, consistent with one break within centromeric alpha satellite. If true, accurate determination of copy number and clinical prognosis are dependent upon molecular assessment with unique clones near the centromere. Variable phenotypes from identical SMCs within families are usually attributed primarily to level of mosaicism, yet these data suggest additional important factors. This new complexity to the origin of SMCs suggests more thorough molecular cytogenetic investigation is necessary to develop genotype-phenotype correlations to improve our prognostic capabilities.

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Origin of structural chromosome rearrangements: Less frequency of paternal origin for unbalanced rearrangements. *E.J. Seo[1,2,3], J.S. Kim[1], J.Y. Lee[1], S.E. Lim[1], E.Y. Choi[1], E.M. Kwon[1], H.W. Yoo[1,2,4].* 1) Medical Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 3) Dept. of Laboratory Medicine, Univ. of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 4) Dept. of Pediatrics, Univ. of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

About 1 in 375 live births has a structural chromosome rearrangement. They might be appeared de novo or originated from a gamete of parent with a chromosome rearrangement. We investigated the origin, frequency, clinical and chromosomal characteristics of structural chromosome rearrangements for the cases in our hospital. Total 132 cases with structural chromosome rearrangements including 54 prenatal and 78 postnatal cases were analyzed. The number for balanced and unbalanced rearrangements respectively were 51 and 81. Seventy-four out of 132 (56.1%) were inherited cases of which paternal origin was 26 and maternal origin was 48. Forty-five of 58 de novo (77.6%) had unbalanced rearrangements while 36 of 74 inherited cases (48.6%) had unbalanced forms. The types of unbalanced rearrangements showed different characteristics between de novo and inherited cases. In cases with balanced rearrangements, the risk for an abnormal phenotype was higher in de novo than inherited cases. Although the number of balanced rearrangements was equal to the parental origin, the number of unbalanced forms was 7 of 26 (26.9%) for paternal origin and 29 of 48 (60.4%) for maternal origin. In summary, the frequency of structural rearrangements was higher in inherited cases than in de novo. For unbalanced rearrangements, the frequency is significantly higher in de novo than in inherited cases (P=0.0017), and interestingly higher in maternal origin than in paternal origin (P=0.0132). This finding suggests that the opportunity to be fertilized with a sperm having an unbalanced rearrangement is less frequent.

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Detection of cryptic chromosomal alterations by genome-wide array comparative genomic hybridization (array-CGH) with 21,600 BAC clones. *B. Nowakowska[1,2], A. Kutkowska-Kazmierczak[2], S.J. Noblin[3], Y.F. Li[1], H. Northrup[3], D. Corzo[4], A. Weise[5], A. Patel[1], E. Bocian[2], S.W. Cheung[1], W.-W. Cai[1].* 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston TX; 2) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 3) Dept. of Pediatrics and Ob/Gyn, The University of Texas Medical School, Houston, TX; 4) Div. of Clinical Genetic, Childrens Hospital, Boston, MA; 5) Institute for Humangenetics and Anthropolgy, Jena, Germany.

Development of array comparative genome hybridization has increased the resolution of detection of chromosome rearrangements more than 50 fold. Genome-wide BAC clone arrays have been developed using chemically modified DNA attached efficiently to an unmodified glass surface (Cai et al. 2002). To minimize clone repeat sequence content, a computer program was applied to select a set of 21,658 BAC clones with unique sequences at both ends. The selected clones have tightly distributed insert size and completely cover the entire human genome. We have identified the cryptic unbalances, subsequently confirmed by FISH analysis. Array-CGH analysis in a 2½-year-old boy with developmental delay, hypotonia, esotropia, paroxysmal alternating torticollis, movement abnormalities, elevated CK values and an apparently balanced de novo translocation t(3;10)(p26.2;q26.3) revealed an additional 3.7 Mb deletion at 10q26.3. An ~1 Mb deletion in 9q22.32 was identified by microarray in a 13-year-old girl with clinical features of Gorlin-Goltz syndrome and a normal 46,XX karyotype. In a third case, an unbalanced 46,XX,add(9)(q34.3) karyotype was found in a child with hypotonia, an anteriorly-placed anus, elevated testosterone, an unusual cry, plagiocephaly and developmental delay. In addition to the loss of the subtelomeric region on 9q, the array-CGH analysis revealed a gain of ~13.2 Mb of 12q24.3 and a gain of the 9q34 region, pending on FISH verification. Overall, we were able to precisely define the sizes of detected deletions or duplications. The results of the array-CGH demonstrate its huge potential for high-resolution, high-throughput molecular karyotyping and precise phenotype-genotype correlations.

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Cytogenetic characterization of Y chromosome structural anomalies in two patients presenting with short stature. *J. Lavoie[1], J. Xu[2], M. Shago[3], R.F. Carter[1], A.A. Khan[4].* 1) Department of Pathology & Molecular medicine, McMaster University, Hamilton, ON, Canada; 2) London Health Sciences Centre, University of Western Ontario, London, Ontario, Canada; 3) Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada; 4) Department of Pediatrics, McMaster Childrens Hospital, Hamilton, Ontario, Canada.

We report the results of cytogenetic studies of structurally rearranged Y chromosomes identified in two patients who were investigated for short stature. Patient 1 was an 18-year-old male referred with a provisional diagnosis of hypochondroplasia. Testing for FGFR3 mutation was normal. Subsequent cytogenetic analysis revealed the existence of an isodicentric Y chromosome for the long arm in all cells analyzed. The isodicentric showed only one primary constriction, which would explain the apparent stability of the dicentric during cell division. The SRY gene was detected using fluorescence in situ hybridization (FISH), consistent with the fact that the patient presented with normal external genitalia. Patient 2 was a 14-year-old boy referred for investigation of mesomelic limb shortening with confirmed Madelung deformity of the forearm on radiographs. G-banding and FISH analysis of lymphocytes revealed a mosaic karyotype with evidence of an unstable ring derived from the Y chromosome. The karyotype of skin fibroblasts was 45,X[6]/46,X,r(Y)[13]. The ring was lacking the long arm heterochromatic region and the tip of the short arm extending from a breakpoint distal to the SRY gene. Further FISH studies are in progess, including assessment for deletion of the SHOX gene located in the pseudoautosomal region on the short arm of the Y; SHOX haploinsufficiency is implicated in Leri-Weill Dyschondrosteosis, which is characterized by mesomelic short stature and Madelung deformity. These cases underline the importance of karyotyping patients with unexplained short stature and to characterize the chromosome constitution as part of the clinical assessment. These results will also be useful in determining whether SHOX haploinsufficiency should be included as a diagnostic consideration for causes of short stature in patients with Y-chromosome anomalies.

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Complex chromosome rearrangements in 17p are associated with genomic architecture involving low-copy repeats. *P. Stankiewicz[1], L.E.L.M Vissers[2], S.A. Yatsenko[1], E. Crawford[3], H. Creswick[4], E.M. Gutter[4], B.B.A. de Vries[2], R. Pfundt[2], C. Marcelis[2], J. Zackowski[4], J.R. Lupski[1], J.A. Veltman[2].* 1) Dept. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Sentara Cytogenetics Laboratory, Norfolk VA; 4) Children's Hospital of the King's Daughters, Norfolk, VA.

Recent data show that complex chromosomal rearrangements (CCRs) are more complex and common than initially thought; however, the molecular mechanisms remain elusive. G-banding and FISH analyses in a 4-month-old boy with developmental delay, hypotonia, growth retardation, coronal synostosis, mild hypertelorism, and bilateral club feet revealed a duplication of the Charcot-Marie-Tooth disease type 1A (CMT1A) and Smith-Magenis syndrome (SMS) chromosome regions, inverted insertion of the Miller-Dieker lissencephaly syndrome (MDLS) region into the SMS region, and the terminal deletion of 17pter. Each of the chromosome breakpoints have been mapped at the BAC clone resolution and the patients karyotype has been designated as: ish der(17)(17qter17p11.2::17p13.317p13.2::17p11.217p13.2::17p11.217p13.2:)*de novo*. Interestingly, the MDLS region was inserted into the SMS LCR, middle SMS-REP. High-resolution genome profiling by tiling resolution array CGH in a 6-year-old girl with mental retardation, short stature, microcephaly and mild dysmorphic features showed a non adjacent ~5 Mb duplication in 17p13.1-p13.2, an ~3 Mb duplication in 17p12 involving *PMP22*, and an ~1.7 Mb duplication in 17p11.2. The duplications were confirmed by MLPA and shown to be *de novo*. *In silico* analysis demonstrated that the proximal breakpoint of the 17p12 duplication maps to the LCR17pA; known to be an unstable genomic region and the breakpoint for uncommon but recurrent SMS deletions. The 17p11.2 duplication is flanked by the middle SMS-REP and the proximal SMS-REP. In both patients, the LCR-evolutionary burden in proximal 17p likely stimulated and mediated the formation of CCRs. We propose that genome architecture involving LCRs may be mechanistically important for the origin of CCRs.

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Replication timing of loci repositioned near centromeric heterochromatin after ring (14) formation. *K. Schlade-Bartusiak, D.W. Cox.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Ring chromosome 14 is associated with growth and mental retardation, characteristic facies and retinitis pigmentosa. Seizures are present in all cases. In our previous study we established that seizures are not due to deletion of a specific locus on one homologue in the preterminal region of 14q. Lack of mosaicism in the studied cultures made mitotic instability of the ring as a main cause of seizures also unlikely. Therefore, the cause of seizures is probably the formation of the ring itself. The new nuclear position of the preterminal region of 14q, together with the local protein environment of centromeric domain could determine different gene activity. The largest deletion detected so far on r(14) encompasses 5 Mb. The region in 14q32 adjacent to the breakpoint contains imprinted genes, as well as genes expressed and not expressed in lymphocytes. The present study focused on the analysis of replication timing (RT), as an approach to study gene expression in this region. Early replicating regions are usually transcriptionally active while late replicating sequences are generally silent. Imprinted regions are characterized by asynchronous replication. We expected r(14) cultures to show significantly changed RT, when compared with controls, as a sign of different gene activity in this region. We used FISH to analyze RT of 1 Mb region of 14q32 in lymphoblast cultures of three r(14) and three controls. We observed a significant level of asynchronous replication throughout the region, in both groups, which was comparable with that of a control imprinted region (SNRPN) in 15q11. Presence of known imprinted genes in the region can explain this observation. RT of a not expressed gene was not significantly different in ring cultures, as compared with controls. However, in ring cultures, we observed slightly delayed RT of a gene that is expressed in lymphocytes. In conclusion, RT of the 14q32 region in r(14) cell cultures is not significantly different from controls. Therefore, gene activity in this region in lymphocytes is either not changed due to ring formation, or changed gene activity is accompanied by only subtle differences in replication timing.

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Cytogenetic Effects of Ethanol in Chronic Alcohol Users. *O. Demirhan[1], D. Tastemir[1], Y. Sertdemir[2].* 1) Medical Biology and Genetics, University of Cukurova, Adana, Turkey; 2) Biostatistics, University of Cukurova, Adana, Turkey.

Alcoholism is a significant public health problem that is also a complex genetic trait, and being relevant to study the induction of chromosomal aberrations by ethanol. The fragile sites (FS) are very interesting subjects for the study of clinical disorders. Still, it is unknown whether individuals with heritable FS have an increased risk of alcohol dependence. However, chromosomal abnormalities may be helpful in identifying disease genes. To search susceptible folate-induced common FS for chronic alcoholics, we have performed chromosomal examinations by using the GTG banding technique for 80 chronic alcoholics and 57 controls. Chromosome rearrangements were observed higher frequency among alcoholics, who revealed a significantly higher frequency of structural and numerical chromosomal aberrations. The most frequent exchange type was inv(9)(p11;q13), 9qh+, and deletion (22)(q13), followed by single cell deletions and aneuploidies. The 22q11 region is involved in chromosomal rearrangements that lead to altered gene dosage, resulting frequently in psychotic symptoms. Two cases with inv(9) (2.5%) and twenty-two cases with 9qh+ (27.5%) detected in this study. This inversion is usually known about 1% in the general population. Although, inv(9) and 9qh+ has been considered to be a normal variant, our observation implies a possible association between the pericentromeric region of chromosome 9 and alcoholism. It is suggested that a susceptibility locus for the alcohol abuse may be located at pericentromeric region of chromosome 9. We also observed a small number of additional karyotypic changes. The FS analyses of the study population provided evidence of genetic susceptibility loci for alcohol dependence on chromosome 1,2,4,3,7,9,5,6,11,12,X,13,14 and 17, in decreasing order. When compared with age matched controls, significant differences in the incidence of FS frequency were detected. But, there were not statistically differences between smoker and non-smoker in healthy control individuals, and smoking as modulating factors had no added effect on the frequency of FS. The results showed that long-term ethanol intoxication can lead to chromosome damage.

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Characterization of the first isotricentric chromosome Y. *J. Xu[1], E. Drozd-Borysiuk[1], J.C. Hsu[1], H. Whitby[1], E. Aston[1], M. Mintz[2], Z. Chen[1], A.R. Brothman[1].* 1) Dept Pediatrics, Cytogenetics, Univ Utah, Salt Lake City, UT; 2) Bancroft Neurosciences Institute, Cherry Hill, NJ.

Tricentric chromosomes are extremely rare events in human beings. We report the detailed molecular-cytogenetic studies of an isotricentric Y chromosome identified in a 23-year old male. The patient was referred for cytogenetic assessment because of aggressive, severe outbursts, large bones and mild facial dysmorphology. He has been diagnosed with " Impulse Control disorder " and has borderline to mild mental retardation with a full scale IQ of 67. FISH and array-CGH revealed an abnormal karyotype: 46,X,itrc(Y)(pterq11.221::q11.221cenq11.221::q11.221pter). ish itrc(Y)(WCPY+,DXYS129++,SRY++,DYZ3+++,DYZ1-,ESTCdy16C07-). arrcgh(Y)(p11.31p11.2)(RP11-400O10RP11-375p13)x2,(Y)(q11.21q11.221)(RP11-350I10RP11-71M14)x4,(Y)(q11.22q11.23)x0. This represents a duplication of Yp, partial quadruplication of Yq (cenq11.221) and partial deletion of Yq(q11.221qter). So far, to our knowledge, no tricentric chromosome Y has been reported in the literature. The formation of this complex rearrangement appears to have involved two separate breakage events, the first resulting in the production of an isodicentric Y chromosome and the second leading to the tricentric isochromosome Y. There was no evidence of mosacism in 50 metaphases and 1000 interphase nuclei scored indicating the stability of the isotricentric Y chromosome. The clinical findings of our case share some common features with other reported cases of tetrasomy Y.

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A deletion on chromosome 11 detected by array CGH in a patient with pulmonary fibrosis: an example of why balanced cytogenetic translocations should be scrutinized. *M. Scholand[1], E. Aston[1], H. Whitby[1], J. Xu[1], R. Selzer[2], T. Richmond[2], N. Singh[1], J. Pell[1], N. Matsumori[1], J. Carey[1], A. Brothman[1].* 1) University of Utah, Salt Lake City, UT; 2) NimbleGen Systems, Inc. Madison, WI.

Pulmonary fibrosis (PF), which results in progressive scarring of the lungs, is a devastating disease which is often fatal despite treatment. While there are environmental risk factors and systemic diseases that are associated with forms of PF, the majority of cases are without a known etiology. Moreover, specific genetic loci associated with pulmonary fibrosis are largely unknown. A woman with a history of pulmonary fibrosis with no known risk factors presented with progressive dyspnea despite aggressive therapy. Conventional karyotyping was performed and this patient was found to have a t(4;11)(q34.2;q21). Characterization of DNA by CGH was done using the Spectral Genomics, Inc. (Houston, TX) SpectralChip 2600, which contains genomic BAC clones spaced at approximately 1 Mb intervals throughout the genome. This analysis detected loss of a single clone at 11q22.1 (RP11-775E2); this was confirmed by FISH using the same clone. Further characterization was then done using a NimbleGen Systems Inc. (Madison, WI) human whole genome CGH array which contains approximately 393,000 oligonucleotides. Probes are masked for repetitive elements and have a median spacing of 6 kb. This high-density tiled approach enables high resolution mapping of both genic and intergenic regions, providing unbiased mapping of DNA breakpoints and copy number polymorphisms. This analysis revealed that the deleted region was 2.6 Mb in size and contained 13 transcripts. Genes within this deleted region may contribute to the pulmonary fibrosis phenotype. CGH array is a powerful technique showing greatly enhanced resolution in the analysis of the human genome. Stepwise analysis by two array platforms allowed for rapid and precise characterization of the region. Several candidate genes associated with pulmonary fibrosis have been identified by this approach. This case demonstrates how further study of an apparently balanced translocation may yield critical information in further understanding disease pathogenesis.

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PURE TRISOMY 4p SUBTELOMERE: AN APPARENT NORMAL FAMILIAL VARIANT. *I. Gadi, L. Wisniewski, S. Griffin, V. Jaswaney, C. Bullen, P. Papenhausen, J. Tepperberg.* Dept Cytogenetics, Laboratory Corp America, Res Triangle Pk, NC.

In our laboratory, the Vysis ToTelvision Multi-Color chromosome specific subtelomere FISH panel has detected various subtelomere rearrangements in 2-4% of patients referred because of idiopathic MR/DD. In a number of these cases, follow up studies demonstrated that apparent unbalanced subtelomere abnormalities in the probands were actually inherited from clinically normal unbalanced carrier parents. We have ascertained 4 unrelated families with an unbalanced derivative chromosome 22 secondary to a translocation between the short arm subtelomere region of chromosome 4 and the short arm of chromosome 22. In each family, FISH testing demonstrated that the breakpoint on 4p was located distally to the Wolf-Hirshhorn syndrome (WHS) critical region. Case 1: B.A. is a 28 yr. old male with idiopathic MR. Case 2: J.A. is a 4 yr. old boy with developmental delay. Case 3: B.S. is a 1 yr. old boy with developmental delay Case 4: H.T. is a 7 yr. old female with asthma, rhinitis, otitis, sleep disturbance and morbid obesity. In two of the 4 cases (case 1 and case 2), parental subtelomere FISH analysis revealed the identical unbalanced rearrangement in a clinically normal parent. Parental FISH studies on case 4 are pending and case 3 was lost to follow-up. Various hypotheses can be offered to account for the observations reported here. First of all, the subtelomeric region of chromosome 4 translocated in these families may not share common breakpoints resulting in trisomy for variable amounts of 4p DNA. Clearly, some of the DNA localized in this region may have no phenotypic expression in a trisomic state and is probably inactive. A more proximal breakpoint, however, may begin to encompass expressed genes. Second, while no imprinted loci have been assigned to 4p, both normal carriers identified were female and the possibility of UPD or another form of regulatory disruption may exist. Finally, it is conceivable that any clinical stigmata observed in the probands may be due to another etiology.

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INVERTED DUPLICATION OF CHROMOSOME 18 SHORT ARM ASSOCIATED WITH MILD PHENOTYPE. *G. Calabrese[1,2], A. Sensi[3], P. Guanciali-Franchi[1,2], E. Morizio[2], D. Fantasia[2], C. Nuzzi[1,2], V. Aiello[3], A. Bonfatti[3], V. Gatta[1], L. Stuppia[1], E. Calzolari[3], G. Palka[1,2], B. Dallapiccola[4].* 1) Scienze Biomed/Genetica Medica, Univ G D'Annunzio, Chieti; 2) Serv. Genetica Umana, Spirito Santo Hospital, Pescara; 3) Genetica Medica, Univ. di Ferrara; 4) CSS-Mendel, Rome, Italy.

Pure 18p duplication phenotype, with absent or mild malformations and mild mental retardation, has been rarely reported (6 cases), and FISH characterization of dup(18p) was performed in only two cases. We report on two unrelated infertile women, aged 36 and 37, respectively, who were referred to cytogenetic units for karyotyping as candidates for IVF treatment. Both patients were single offspring with normal auxologic parameters and menarche at 12 yrs with normal rhythm. The older patient presented with borderline intelligence. Physical examination disclosed mild common dysmorphisms: high palate, micrognathia, long philtrum, and bulbous tip of the nose. Parents were apparently normal. Both patients showed a diploid karyotype with an add(18)(p11.3) de novo. FISH analysis with a chromosome 18 painting probe showed a complete decoration of rearranged chromosome. FISH with alphoid D18Z1 probe unraveled a signal on the tip of the short arm of the der(18) in addition to the normal signals at both chromosome 18 centromeres. MultiTelomere FISH test disclosed chromosome 18p subtelomere deletion on der(18) short arm in which no other subtelomeric region was found. FISH with a panel of RP11 BAC clones mapped at both telomere and centromere breakpoints detected duplicated regions on der(18) in addition to a single signal on normal chromosome 18. FISH experiments on interphase nuclei displayed that duplicated distal clones hybridized closer each other than more proximal clones, confirming the presence of an inverted duplicated segment estimated 13 Mb in size. Although inv dup(18p) is possibly underreported because of the normal or near normal phenotype, an accurate prognosis remains difficult when this anomaly is incidentally found de novo at prenatal diagnosis. Molecular characterization of a larger series of patients could provide better classification and prognostic information for recurrent inv dup(18p) abnormalities.

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A recombinant chromosome 14 derived from 2 maternal inversions: Delineation of a complex karyotype by high resolution comparative genome hybridization. *B. Levy[1,2,3], O. Nahum[2], R.D. Clark[4].* 1) Dept Human Gen,Mt Sinai Medical Ctr, New York, NY; 2) Dept Pediatrics, Mt Sinai Medical Ctr, New York, NY; 3) Dept Obstetrics, Mt Sinai Medical Ctr, New York, NY; 4) Division of Medical Genetics, Dept Pediatrics, Loma Linda School of Medicine, Loma Linda, CA.

We report a case of a complex recombinant chromosome 14 derived from a mother with two different pericentric inversions in chromosome 14. This dysmorphic male infant weighed 1768 g at term. Dysmorphic features were large fontanelles, hypertelorism, micrognathia, large downturned mouth, clinodactyly, adducted thumbs, overlapping digits, undescended testis. He had an irritable high-pitched cry, absent gag, incomplete Moro and weak suck. Amniocentesis for IUGR and abnormal MSAFP showed an apparent proximal duplication of 14p10-14p12, a distal duplication of 14q31-14q32.3 and a distal deletion from 14pter to 14p13. The mother was mosaic with two cell lines: 46,XX (78%) and a cell line (22%) that contained a different pericentric inversion in each chromosome 14: inv(14)(p11.2q13) and inv(14) (p12q31).

High-resolution comparative genomic hybridization (CGH) on the patient indicated a gain of chromosomal material in two places on the long arm of chromosome 14 at 14q13-14q21.1 and 14q31-14qter. This was confirmed by FISH using the sub-telomeric 14q probe and multiple BAC probes, which indicated that proximal 14q11.2-q21.1 (containing one of the duplicated regions), was inverted and inserted into the short arm. The apparent loss of 14pter-p13 was reconciled in the GTG image but was not detected by CGH as this region is suppressed using Cot-1 due to its repetitive nature. Based on the GTG image, CGH and FISH results, the orientation of the derivative ch14 was qterq31::q21.1q11.2::p11.1q11.2::q13qter. Possible mechanisms by which this complex derivative was formed will be reviewed. We conclude that high resolution CGH can be an important tool for the accurate delineation of complex derivative chromosomes.

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Definition of a deletion phenotypic map of chromosome 14 to search for gene-disease in the ring 14 syndrome. *L. Seminara[1], G. Matarrelli[1], G. Gobbi[2], E. Della Giustina[3], A. Scarano[3], G. Neri[1], M. Zollino[1].* 1) Genetica Medica, Università Cattolica , Rome, Italy; 2) Unità Ospedaliera NPI Ospedale Maggiore Pizzardi, Bologna, Italy; 3) Struttura Complessa Neuropsichiatria Infantile Ospedale Santa Maria Nuova, Reggio Emilia,Italy.

The ring 14 syndrome is a rare condition with mental retardation, epilepsy, retinal anomalies and craniofacial dysmorphisms. It is caused by rearrangements of chromosome 14 to form a ring, usually associated with 14q deletions of variable extent. Clinical manifestations are only in part justified by the terminal 14q deletion. Positional effects, mitotic instability of the ring and mosaicism with a monosomic cell line can also be responsible for some clinical manifestations. With the purpose of looking for gene-disease in the ring 14 syndrome, we tried to define a deletion phenotypic map of chromosome 14, by means of a clinic-genetic analysis of a total of 12 families with different chromosome rearrangements, including ring (14) (6 cases de novo and 1 familial); interstitial deletions (total 4) affecting 14q11.2, 14q12, 14q24.3q32.12 and 14q31q32 regions, respectively, and one balanced translocation t (10;14) (q25.3; q12). Genetic analysis was performed by conventional cytogenetics, by FISH with a total of 61 molecular probes specific for chromosome 14q and by microsatellite segregation analysis (to look for parental origin of the rearrangement). Relevant results are about: 1) familiarity; 2) genotype-phenotype correlations with respect to degree of mental retardation, facial characteristics, epilepsy and retinal anomalies. 3) Comparative analysis of cases with ring 14 and cases with interstitial deletions of different 14q regions led us to tentatively map some clinical signs to very restricted chromosome regions. Supported by International RING 14 Association (info@ring14.com).

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Interstitial duplication 8q 22.2-24.3 in a patient with congenital malformations and normal psychomotor development. *D. Concolino[1], M.A. Iembo[1], M.T. Moricca[1], E. Rossi[2], R. Marotta[3], P. Strisciuglio[1].* 1) Department of Pediatrics, University of Catanzaro,Italy Catanzaro, Italy; 2) Department of Human Genetics, University of Pavia, Italy; 3) Department of Psychiatry, University of Catanzaro, Italy.

We report a new case of de novo partial duplication of 8q in a child with short stature, facial dysmorphisms and heart malformations. The clinical signs associated to 8q duplication are variable and correlated with the size of duplicated segment. The dysmorphic signs include: broad face, downslanting palpebral fissures, hypertelorism, depressed bridge and anteverted tip of the nose, long philtrum, micrognathia, small and posteriorly rotated ears, cryptorchidism, clinodactyly. Almost all had heart malformations. Mental deficiency is severe with frequent occurrence of seizures. PG is second child of healthy and unrelated parents; the family history, the pregnancy and the delivery were unremarkable. Physical examination at age of 6 months of age showed weight and length < 5 centile, typical facial dysmorphic features, frontal epydermoid, clinodactyly, cryptorchidism. The echocardiography showed atrial and ventricular septal defects. The neurological examination and the evaluation of psychomotor development at 3 years of age were normal. No other malformations were present. Chromosome analysis showed 46,XY,dup(8)(p22-tel) de novo. The subtelomeric probes specific for chromosome 8 showed normal signals in both telomeres, so the duplication is interpreted as direct interstitial duplication. Molecular analysis by FISH was performed and the duplicated region was included from 8q 22.2 to 8q24.3 (from 100Mb at 144.3 Mb). Recently a de novo 2.3 Mb inverted duplication of 8q24.3 in a subject with profound psychomotor retardation and epilepsy has been described suggesting that small subtelomeric de novo duplications may be responsible for mental retardation. Our patient presents mild phenotype with normal mental development, although the presence of a large direct interstitial duplication 8q. The absence of subtelomeric regions duplication in our case could be explain the absence of psychomotor retardation in this patient.

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Parental 4p16.3 inversion polymorphism is not a risk factor for WHS-associated genomic rearrangements. *M. Zollino, M. Murdolo, R. Lecce, I. Mancuso, D. Orteschi, G. Marangi, G. Neri.* Dept Medical Genetics, Univ Catt Sacro Cuore, Rome, Italy.

Wolf-Hirschhorn syndrome (WHS) is a MR/MCA contiguous gene syndrome, resulting from partial 4p deletion. Genomic rearrangements represent de novo events in most cases. Although they are expected to be isolated deletions, there is increased evidence of their complexity. In addition, the role of a parental 4p16.3 inversion polymorphism is still unclear. A total 61 WHS families were analysed both clinically and genetically. Genetic analyses included: a) conventional and molecular cytogenetics (a total of 70 molecular probes 4p-specific and all telomeres); b) microsatellite segregation analysis, c) multiple colour FISH (detection of 4p16.3 inversion polymorphism). We found that a) the basic genomic rearrangements were de novo events in 55 out of 61 patients (90%); de novo rearrangements were isolated deletions in 44/55 patients (80%), unbalanced translocations in 7 (12,8%), consisting of t(4p;8p) (5 cases), t(4p;11p) (1 case), t(4p;7p) (1 case); dup/del(4p) in 3 (5,4%); double intrachromosomal rearrangement in one (1,8%). A total of 29 families were simultaneously tested for type of rearrangement, parental origin and parental 4p16.3 inversion polymorphism. Rearrangements were paternal in origin in 22 out of 29 cases (76%). Nearly the totality of them were isolated deletions (21/22). The 4p16.3 inversion polymorphism was absent in both parents in each case. Genomic defects were maternal in origin in the remaining 7 cases (24%), of which four were de novo unbalanced t(4p;8p), one t(4p;7p) and two isolated deletions (one interstitial, one terminal). The 4p16.3 inversion polymorphism was detected in the mother in 3 out of 7 cases of maternally derived rearrangements. They all were t(4p;8p). It was absent in both parents in one case of t(4p;8p), in the two isolated deletions and in the t(4p;7p). We conclude that the 4p16.3 inversion polymorphism is not a risk factor for WHS-associated genomic rearrangements. Supported by Telethon, grant GGP030253.

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A Centromeric Translocation Diagnosed by FISH. *A. Zaslav[1], J. Jacob[1], R. Kazi[1], S. Allan[2], K. Probherbs[3], J.E. Fox[2].* 1) Dept Laboratory Medicine, Long Island Jewish Medical Ctr, New Hyde Park, NY; 2) Department of Pediatrics, Schneider Childrens Hospital, The Long Island Campus of The Albert Einstein College of Medicine, New Hyde Park, NY; 3) Department of OB/GYN, Antepartum Testing Unit, Long Island Jewish Medical Center, The Long Island Campus of The Albert Einstein College of Medicine, New Hyde Park, NY.

Centromeric translocations have been extensively reported. However, these translocations have rarely been ascertained by FISH alone. Here we report a prenatal case with this type of translocation. Amniocentesis was performed on a 40-year-old African American woman for AMA and late gestational age (21 weeks). Direct interphase analysis using the Aneu Vysion X/Y/18cen TM probe and the LSI 13/21 probe (Vysis, Downers Grove, IL) revealed a signal pattern consistent with one normal X centromere signal, one X centromere signal half the size of the normal X, and one normal Y centromere signal. FISH results were ambiguous and cytogenetic analysis was expanded to rule out the presence of a ring X, mar X and/or sex chromosome mosaicism. Patients were counseled to wait for the cytogenetic results to definitively determine the karyotype of the fetus. Cytogenetic analysis revealed a normal 46,XY male karyotype in 50 metaphase spreads from five in situ culture vessels. FISH was repeated on 10 metaphase spreads. The above probes were used and revealed a signal pattern consistent with one X centromere signal, one Y centromere signal, and one 19 centromere signal hybridizing with the X centromere probe. Parental chromosome analysis and FISH revealed that this was an apparently balanced paternally inherited reciprocal translocation [i.e., t(X;19)(p10;q10)]. A full term infant was delivered and had normal physical findings. To our knowledge, this is the first report of a centromeric translocation ascertained by direct prenatal FISH interphase analysis. This finding reinforces the policy that FISH analysis should not be used as the basis for irreversible therapeutic action. This case illustrates the importance of reporting unusual FISH results in conjunction with standard cytogenetic analysis for patient management and genetic counseling.

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Comprehensive analysis of WHS using CGH microarray. *S.T. South[1], J.C. Carey[1], H. Whitby[1], A. Battaglia[1,2], A.R. Brothman[1].* 1) Dept Peds/Div Med Genetics, University of Utah, Salt Lake City, UT; 2) Inst of Neurology & Psych, Stella Maris Clinical Research Inst, Univ Pisa, Pisa, Italy.

Wolf-Hirschhorn syndrome (WHS) is a multiple malformation syndrome with a broad range of clinical manifestations. The minimal diagnostic criteria include mild to severe mental retardation, hypotonia, growth delay, and a distinctive facial appearance. Variable clinical manifestations include severe feeding difficulties, seizures, antibody deficiency, and major congenital anomalies such as skeletal anomalies, heart lesions, oral facial clefts, sensorineural deafness and genitourinary tract defects. WHS is caused by deletions involving chromosome region 4p16.3. Clinical variation may be explained by variation in the size of the deletion. However, in addition to having a deletion involving 4p16.3, at least 15% of WHS patients are also duplicated for another chromosome region due to an unbalanced chromosome translocation. Therefore, we hypothesize that some of the variation in clinical phenotypes may be due to the additional material from another chromosome. Furthermore, it is likely that the prevalence of unbalanced translocations resulting in WHS is underestimated since many unbalanced translocations can be missed using conventional chromosome analyses such as karyotyping and standard FISH. In contrast, CGH microarray will comprehensively evaluate the entire genome at a high resolution for unbalanced rearrangements, including unbalanced translocations. Using CGH microarray we are conducting a study to access the prevalence and associations of unbalanced translocations among WHS patients. Within the first six months of this study we have enrolled 15 participants with WHS in the study. Thus far, CGH microarray has been completed on 7 patients and 3 of these patients have a derivative chromosome 4 due to an unbalanced translocation. Results of each participants genomic analysis and comparison of their clinical manifestations will be presented.

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Prenatal diagnosis of heterokaryotic monozygotic twins discordant for Turner syndrome in a recurrent familial dizygotic twin history. *M. Gadji[1], K. Krabchi[1], M. Ferland[1], M. Bronsard[1], A. Bastide[2], J.L. Ardilouze[3], C. Caron4[4], A. Langevin[4], R. Drouin[1].* 1) Serv. of Genetics, Dept of Pediatrics, Fac. of Medicine and Health Sciences, Univ. de Sherbrooke, Sherbrooke, Québec, Canada; 2) Department of Obstetrics and Gynecology, CHUL, University of Laval, Quebec, Canada; 3) Service of Endocrinology, Department of Medicine, Faculty of Medicine and Health Sciences, University of Sherbrooke, Québec, Canada; 4) Lac St-Jean Clinic of Obstetrics and Gynecology. Alma, Québec, Canada.

Turpin et al. (1961) reported the first case of heterokaryotic monozygotic dichorionic twins. We describe a similar set of monozygotic twins, one of whom presented a typical Turner Syndrome (TS) phenotype (twin 1) and the other a normal male phenotype (twin 2). Fetal ultrasonography at the 14th week of gestation showed a dichorial diamniotic pregnancy with a hygroma colli and other features associated with TS in twin 1 and no anomalies in twin 2. FISH (Fluorescent In Situ Hybridization) analyses performed on amniocytes showed a 45,X[70]/46,XY[30] mosaicism in twin 1 and a normal XY-male chromosome modality in twin 2. Whereas cultured amniocytes showed an homogeneous 45,X [100] karyotype in twin 1 and a normal 46,XY [100] karyotype in twin 2. At birth, twin 1 presented a typical TS phenotype and t win 2 had a normal male phenotype. Postnatal molecular (FISH) and conventional cytogenetic investigations of blood lymphocytes showed the same normal 46,XY chromosome constitution in both twins. However, the same approach performed on fibroblasts showed 100% of 45,X in twin 1 and 100% of 46,XY in twin 2. Monozygosity was confirmed by molecular analysis using a set of microsatellite markers of different chromosomes with DNA extracted from both lymphocytes and fibroblasts. The mechanism of this case could be explained by two events arising in the primary blastomeric step: one leading to a XY constitutional monozygotic twins (probably in the first day) and the second event, to the loss of the Y chromosome during mitotic division of blastocytes in twin 2. The rarity of heterokaryotic monozygotic twins in a familial history of dizygotic twins constitutes the novelty of this case report.

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Independent mechanisms leading to two different sex chromosome deletions in one individual. *K. Hovanes[1], R. Falk[1], D. Jeandron[3], M. Karantza[2], M. Geffner[2,3], R. Schreck[1].* 1) Medical Genetics Institute, Cedars Sinai Medical Center, Los Angeles, CA; 2) Endocrinology, Diabetes, and Metabolism, Childrens Hospital Los Angeles, Los Angeles, CA; 3) The Saban Research Institute of CHLA, Los Angeles, CA.

A 13.5-yr-old boy referred for short stature had a generalized scaly rash. A similar rash was reported in male, maternal relatives, but no other reports of short stature. Height was 142.2 cm (-3.0 SD) (mid-parental target was 178.7 cm). Examination revealed Tanner Stage (TS) 1 pubic hair (PH). Bone age was age appropriate. Radiological exam revealed short 4^{th} and 5^{th} metacarpals. Testing showed normal hormone levels, chemistry panel, and urinalysis. A skin biopsy was consistent with ichthyosis. Six month follow-up showed height velocity of only 3.75 cm/yr but advance to TS2 PH. Molecular analysis of the *SHOX* gene revealed lack of heterozygosity at 27 intragenic SNPs, consistent with a deletion of the entire *SHOX* gene. The method used could not determine the chromosomal origin of the deletion. No mutations were found in the remaining allele. Thus, short stature was due to *SHOX* haploinsufficiency. The presence of both X-linked ichthyosis due to *arylsulfatase C (STS)* deficiency and a confirmed *SHOX* deletion suggested a contiguous gene deletion at Xp22.3. Cytogenetic analysis showed deletions on both his X and Y chromosomes. Karyotype was 45,X[6]/46,X,idic(Y)(p11.3)[14] with two different cell lines, one with an isochromosome of the Y and one which had lost the abnormal Y. FISH demonstrated the presence of the *SRY* gene on the isodicentric Y and deletion of the *arylsulfatase C* gene on the X chromosome. FISH analysis using two RP11-BAC clones, 91D5 and 132H11, 0.1 Mb and 0.9 Mb telomeric and centromeric to *SHOX* respectively, showed the *SHOX* gene present on the X chromosome but deleted from isodicentric Y chromosome. Thus, the phenotype is the result of a maternally inherited deletion of the X chromosome and a presumed de novo rearrangement of the Y chromosome. The additional information gained from cytogenetic analysis demonstrates that the confluence of rare events can explain an unexpected finding.

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Array-CGH reveals a gain of proximal 15q associated with autism in a multiplex family with a familial cryptic translocation t(14;15)(q11.2;q13.2). *M. Koochek[1,5], C. Harvard[2,5], MJ. Hildebrand[1,5], M. VanAllen[1], JJA. Holden[3,4,5], MES. Lewis[1,5], E. Rajcan-Separovic[2,5].* 1) Depts. of Medical Genetics &; 2) Pathology, University of British Columbia, Vancouver, Canada; 3) Depts. of Physiology &; 4) Psychiatry, Queen's University, Kingston, Canada; 5) The Autism-Spectrum Disorders -Canadian American Consortium (ASD-CARC; www.autismresearch.ca).

Autism is a neurodevelopmental condition with a strong genetic etiology. In ~1% of cases, duplication of the 15q11-13 region is reported and most often it is due to interstitial duplication or supernumerary marker chromosomes 15. Here we report array-CGH, cytogenetic and clinical evaluations of a family with autism and at least two generations of a maternally inherited gain of 15q11-13 occurring as a result of abnormal segregation of a cryptic familial translocation with breakpoints at 14q11.2 & 15q13.2. The translocation was identified using the 1Mb resolution whole genome array (Spectral Genomics), as the affected family members had normal karyotypes. There was a gain of 7 clones from proximal 15q, loss of 2 clones from proximal 14q and gain of 2 clones from 6q. FISH analysis with chromosome 14 & 15 clones combined with DAPI reverse banding of the affected members showed an abnormal karyotype with one normal copy of 15 and the der(15)t(14;15)(q11.2;q13.2) resulting in the gain of proximal 15 and loss of proximal 14q. Two-color FISH using probes for 14qter and centromere 15 confirmed carrier status in the mothers of our probands, and ruled out this cryptic translocation in unaffected family members. The duplication of 2 clones from 6q in the affected subjects was familial in origin and detected via the array analysis. Our findings suggest that the gain of 15q in autism may be due in some cases to cryptic translocations with breakpoints in the pericentromeric regions of chromosome 15 and a different acrocentric chromosome. Close inspection of pericentric regions of all acrocentric chromosomes is therefore warranted, and any variation in their size may justify parental chromosome analysis and FISH analysis of the autistic proband using probes from the 15q proximal region as an important means of autism recurrence risk determination.

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X CHROMOSOME ORIGINATES THE MAJORITY OF MARKERS AND RINGS CHROMOSOMES IN TURNER SYNDROME. *M.Y. Nishi, M.A. Medeiros, M.F.A. Funari, C. Rossi, A.E.C. Billerbeck, B.B. Mendonca.*
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Introduction: The absence of genetic sequences of one of the sexual chromosomes is the cause of gonadal dysgenesis in Turner syndrome (TS). Around 6% of TS patients present a marker or a ring chromosome in their karyotype, whose chromosomal origin is rarely identified by classical cytogenetic analysis. Several studies, using molecular biology techniques, demonstrated that the marker/ring chromosomes (MRC) are derived from the X or Y chromosomes. The Y chromosome sequences in dysgenetic gonads are related to gonadal tumor development, especially gonadoblastomas, therefore prophylactic gonadectomy is indicated.

Aim: Identify the origin of MRC in TS patients using FISH or PCR techniques.

Material and Methods: Forty-nine patients, aged from 6 to 41 years old, were studied, 19 by FISH and 30 by PCR. Twenty-eight patients have a cell lineage with at least one marker chromosome and 21 with a ring chromosome. Genomic DNA was extracted from peripheral blood leukocytes and amplified with specific primers for 3 different pericentromeric *loci* of Y chromosome. The metaphase chromosomes were obtained from peripheral blood lymphocytes cultures nd hybridized with centromeric probes for the Y and X chromosomes (Vysis Inc.,Downers Grove, USA).

Results: FISH revealed that 15 MRC were derived from the X and 3 from the Y chromosome; in one patient the probes did not hibridize to the marker chromosome. PCR showed that the MRC were originated from the Y chromosome in 4 patients.

Discussion and Conclusion: Both techniques revealed that the majority of MRC in TS patients were originated from the X chromosome (86%) and not from the Y chromosome, therefore gonadectomy would not be indicated in these cases. Both techniques are good for such identification; however, FISH showed to be a faster and easier method, but more costly than PCR, whereas PCR showed a higher sensitivity compared to FISH.

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De novo unbalanced cryptic chromosomal rearrangement involving subtelomeric regions 2q37 and 20p13 in a child with multiple congenital anomalies and dysmorphic features : Genotype phenotype correlation and literature review. *S.K. Murthy[1], A. Faquih[2], S. Mani[1], S. Naveed[1], A.K. Malhotra[1], A.I. Al-Khayat[3], M.T. AlAli[1].* 1) Department of Genetics,Al Wasl Hospital,DOHMS,Dubai, PO Box 9115, United Arab Emirates; 2) Department of Neonatology; 3) Department of Paediatrics.

We present here our cytogenetic observations in a one month old female child with dysmorphic features. The child presented with : odd facies, micro-opthalmia, epicanthal folds, low hair line, large and flat right ear, low set ears, high arch palate, arachnodactyly, hypotonia, cardiac murmer, left multicystic dysplastic kidney. Chromosome analysis by G-banding showed the presence of a derivative chromosome 2 with a cryptic chromosomal rearrangement involving 2q terminal region. FISH studies using ToTelVysion multicolor FISH probe (Vysis) showed deletion of 2q subtelomere and addition of 20p subtelomere at 2qtel in addition to the two normal chromosome 20s. Chromosome analysis and FISH studies of the parents showed normal chromosome complement. The final karyotype was interpretted as 46,XX,der(2).ish der(2)t(2;20)(q37;p13)(2ptel+,2qtel-,20ptel+) de novo, leading to a monosomy of 2q tel and trisomy of 20p tel in the patient. Common clinical findings as reported in the literature involving these two chromosomomal breakpoints include - development delay, macrocephaly, frontal bossing, depressed nasal bridge, cardiac anomaly, hypotonia and repetitive behavior/autism. Our patient shows several features corresponding to 2q37 deletion as well as of Allagile syndrome. Very rarely Allagille syndrome has been reported in patients with trisomy 20p with distinctive facies, cardiovascular anomalies, paucity of interlobular bile ducts, ocular anomalies and minor skeletal malformations. Further molecular studies would help to delineate the exact breakpoint and genes involved in the clinical manifestation of our patient. To our knowledge, this is the first report with such a rearrangement. Since complex cryptic rearrangements are extremely rare, our patient is of great interest to help us better understand the underlying genetic causes and its correlation with the clinical manifestations.

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Efficiency of small CGH-array targeting regions between segmental duplications in detecting pathologic and polymorphic cryptic chromosomal imbalances. *I. Cusco[1], E. González[2], M. Vilardell[1], O. Villa[1], B. Rodriguez[1], X. Estivill[2], L. Sumoy[2], L.A. Pérez-Jurado[1].* 1) Unitat de Genetica, Univ Pompeu Fabra, Barcelona, Spain; 2) Centre de Regulació Genòmica, Barcelona, Spain.

Five to ten percent of the human genome consists of large blocks (5-400kb) of highly identical (95%) low copy repeats (LCRs) also called segmental duplications. These LCRs appear to have an important role in the dynamic evolution of chromosomes and can mediate chromosome rearrangements underlying genomic disease or genomic polymorphism (deletions, duplications or inversions) through non-allelic homologous recombination. We have constructed a microarray including 228 BACs covering single-copy regions flanked by LCRs and all the subtelomeric regions. Using this CGH-array, we have screened a collection of 94 samples of cytogenetically normal patients with a variety of conditions to look for candidate regions of sub-chromosomal aneusomy. Patient-specific rearrangements likely responsible for the disease were detected and confirmed in 6 samples (6.4%): two cases of Silver-Russell syndrome (dup~10Mb 7q36-qter/del~8Mb 10q26-qter; dup1Mb 7q14), one polymalformative syndrome (del~0,5Mb 18q23-qter/dup~3Mb 8p23-pter), and three with mental retardation and autistic features (dup~3Mb 15q12; del~0,5Mb 15q11; dup~4Mb 10q23). We also detected 61 abnormal signals (21 deletions, 8 duplications and 32 deletion/duplications) present in 2-9% of the samples that might correspond to copy number polymorphisms (CNPs). Almost half of these presumed CNPs (28/61) have also been described elsewhere (Iafrate 2004, Sebat 2004, Bejjani 2005, Sharp 2005). Alternative methods (FISH, MLPA, microsatellites) allowed us to confirm only half of the putative CNPs tested (n=12), suggesting false positive results of the array. Confirmation of the novel variable loci not previously reported is in process. Array-CGH is a powerful tool to detect sub-microscopic pathogenic rearrangements in patients as well as CNPs, although careful analysis and confirmation by other methods may be required due to some false positive results. The strategy of targeting putative hotspots located between LCRs has proven to be highly effective.

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Routine MLPA screening for telomeric rearrangements : a low cost, high throughput substitute for multiFISH technology. *S. Drunat[1], A. Aboura[2], A.C. Tabet[2], O. Dupuy[2], C. Baumann[3], J. Elion[1], A. Verloes[3,4].* 1) Molecular Genetics Unit, Genetics Dept, Robert DEBRE Hospital, Paris, France; 2) Cytogenetics Unit, Genetics Dept, Robert DEBRE Hospital, Paris, France; 3) Clinical Genetics Unit, Genetics Dept, Robert DEBRE Hospital, Paris, France; 4) INSERM U676.

Cryptic telomeric rearrangements (CTR) are found in 5 % of patients with idiopathic mental retardation (MR) or Multiple Congenital Anomalies/MR syndromes, and apparently normal karyotype. Despite their clinical relevance, multiFISH screening remains difficult to apply widely, because kits are expansive and methodology extremely time consuming. Clinical presection criteria as De Vries scale are unsatisfying. Multiplex ligation-dependent probe amplification (MLPA) is a highly sensitive and rapid alternative to multiFISH. It can be used on any DNA source (blood, amniocytes, CVS, paraffin-embedded tissue...) The method allows batch screening of 20 patients per run. We used 2 commercial sets of probes, the SALSA P036B and P70 human telomere kits, to assay retrospectively patients with known dup/del syndromes. All were detected and, more importantly, there was no false negative tests. We will present results of MLPA to a series of more than 500 systematically collected patients referred to the genetic clinic for evaluation of MR or autism. In all cases with abnormal/dubious MLPA, confirmatory pantelomeric FISHing was performed. Detailed results of this ongoing study will be presented. Preliminary results (at the time of submission,) in a prospectively collected series of 240 samples: we were able to confirm 6 anomalies. Three further MLPA positive but FISH-negative cases were considered as polymorphisms. Nine further MLPA-positive cases are pending. Conclusion: subtelomeric MLPA screening can be done without preselection, and appears to be cost-effective, time saving, and clinically rewarding, as some of the positive patients would not have been selected on clinical grounds for multiFISH. Moreover, the drastic reduction in FISH-based screening demand allows buzy diagnostic laboratories to re-affect time to refining the cytogenetics/molecular definition of positive cases.

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A locus for ano-sacral malformation maps to 6q25.3 in a 0.3 Mb interval region. *L. Titomanlio[1,2], I. Giurgea[3], M. Elmaleh[4], Ph. Sachs[2], F. Chalard[4], O. Dupuy[5], A. Verloes[1,6].* 1) Clinical Genetics Unit, Dept of Genetics, Robert DEBRE Hospital, Paris, France; 2) Dept of Child Neurology,Robert DEBRE Hospital, Paris, France; 3) Dept Biochemistry & Genetics and INSERM U654, Henri Mondor Hospital, Creteil, France; 4) Dept Medical Imaging, Henri Mondor; 5) Cytogenetics Unit, Dept of Genetics, Robert DEBRE Hospital, Paris, France; 6) INSERM U676.

Partial absence of the sacrum is a rare congenital defect which also occurs as an autosomal dominant trait; whereas imperforate/ectopic anus is a relatively common malfrormation, usually observed in polymalformative syndromes. We report on a girl born to healthy consanguineous parents (first cousins once removed). She was immediately admitted to a tertiary care center for surgical correction of an anal imperforation with an associated recto-vaginal fistula. At 3 years of age, imaging studies showed partial sacral agenesis and a tethered cord. Facial dysmorphism included a high forehead, epicanthal folds, downslanting palpebral fissures, hypertelorism, a depressed nasal root. Brain MRI showed a bilateral opercular dysplasia with an unilateral (right) pachygyria; MRI and X-ray imaging of the spine disclosed a tethered cord associated with a partial sacral agenesis. She showed a global and moderate developmental delay. Ophthalmologic examination evidenced bilateral microphtalmos (<20 mm) and relative microcornea. Terminal deletions of 6q (6q25à6qter) have been rarely reported in the literature and have been associated to a specific phenotype. Cytogenetic studies in our patient disclosed a pure de novo 6q25.3àqter deletion. By genotype analysis, we detected in our patient a maternal allele loss for the D6S1581, D6S264, D6S446 markers. Distal 6q deletions have been frequently reported in association with sacro-anal malformations (sacral agenesis, anal imperforation/ectopy). By comparing the reported cases of affected patients carrying a pure terminal 6q deletion, we delimited a critical region spanning 0.3 Mb. Thus we hypothesize that there is a gene in the deleted region whose haploinsufficiency impairs the normal development of these structures, possibly acting on the notochordal development.

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Application of Whole Genome Amplification to Chromosomal Microarray Analysis for Genetic Disorders. *D. del Gaudio, T. Sahoo, J. Li, SH. Kang, P. Ward, CM. Eng, A. Beaudet, SW. Cheung, BB. Roa.* Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Molecular cytogenetic techniques have improved the diagnosis of chromosomal abnormalities. Comparative genomic hybridization on microarrays has demonstrated utility for detecting clinically relevant DNA dosage variations at high resolution, sensitivity and speed. We have developed a clinical microarray at Baylor College of Medicine that encompasses loci involved in over 55 genetic disorders, plus clones for 41 relevant sub-telomeric regions. Application of chromosomal microarray analysis (CMA) to prenatal testing is being evaluated. Limitations of DNA extracted directly from uncultured amniocytes and chorionic villi preclude rapid testing. Yields are improved by using fetal cell cultures, but this delays prenatal testing and reporting. One option is to incorporate whole genome amplification (WGA) into the CMA process. The WGA technology involves random fragmentation of genomic DNA and adapter ligation followed by PCR amplification with universal primers. We used a WGA kit developed by Rubicon Genomics and subsequently released by Sigma Aldrich. Our initial validation included 6 whole blood genomic DNA samples tested in parallel with the corresponding WGA products. CMA testing showed comparable results, with no significant amplification bias observed for genomic clones included in the microarray. This approach was then evaluated for prenatal testing by CMA analysis. Genomic DNA extracted from 35 prenatal samples (27 direct amniocytes and 8 direct CVS samples) were subjected to WGA. Comparable results were obtained on parallel CMA testing of fetal genomic DNA samples and corresponding WGA products, including positive controls. Results were usually obtained in 6 days or less. Comparative PCR analysis of multiple polymorphic STR loci ruled out maternal cell contamination on fetal DNA and WGA samples. We observed 100% concordance in this study on CMA analysis using genomic DNA and the corresponding WGA products. The use of WGA provides a viable approach to facilitate rapid prenatal testing on direct fetal samples.

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Paucity of primary identification of new cryptic subtelomere rearrangements with subtelomere FISH: a three year retrospective analysis. *H.M. Saal, L. Bao, E.K. Schorry, R.J. Hopkin, N.D. Leslie, T.A. Smolarek.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

A review of the literature shows that in many cytogenetics laboratories the frequency of anomalies identified by subtelomere FISH panel ranges from 3-7%. The experience in our institution has not produced similar results; although, indications for testing have closely matched those reported in the literature. In the past three years we have performed 233 subtelomere FISH analyses, of which there were only five subtelomere abnormalities diagnosed (2.1%) which were not initially identified by cytogenetic analysis, usually high resolution. Significant among these five cases was one patient who had a 14q deletion which was also seen in the phenotypically normal father and a 22q deletion in which the FISH study was performed prior to completion of the chromosome study where the deletion was identified microscopically. Therefore, in only three of 233 cases (1.3%) were subtelomere studies essential for identifying pathologic chromosome abnormalities. Utilization of subtelomere FISH panels is a helpful adjunct to cytogenetic analyses; however, this technology should not be considered a replacement for high resolution chromosome studies, since interstitial deletions or duplications may also be missed. During the same period we have identified a greater number of interstitial rearrangements (24) than subtelomere rearrangements, many of which were very subtle. These data underscore a need for continued emphasis on high quality microscopic analysis in addition to continued development of new technologies, including comparative genome hybridization, for enhancing cytogenetic diagnosis.

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CELL CYCLE DURATION IN A SECKEL SYNDROME PATIENT. *L. Bobadilla-Morales[1], J.R. Corona-Rivera[1], I. Cervantes-Luna[1], C.E. Monterrubio-Ledezma[1], R. Iñiguez -Rodruiguez[1], A. Corona-Rivera[1,2].* 1) Lab de Citogenetica Genotoxic, Univ Guadalajara, CUCS, IGH, Guadalajara, Jalisco, Mexico; 2) Unidad de Citogenética, Hospital Civil de Belen Fray Antonio Alcalde,, OPD.

Seckel syndrome has been proponed as one chromosome instability syndrome. This nosological group includes genetic entities sharing short stature, developmental delay, immunodeficiency, high cancer risk, and autosomal recessive inheritance. This group includes also defects in the ways of DNA reparation and consequently spontaneous or induced breackages and chromosome instability. It was proposed that chromosome instability in this syndrome can be related to cell cycle impairement or apoptosis leading to reduction of cells in the organism and failure to thrive. Considering that altered cell cycle can be involved in Seckel Syndrome we performed cell cycle measurements in one patient with Seckel syndrome and paired control. Cellular proliferation kinetics was measured by the method called average generation time by differential sister chromatid stain of chromosome spreads from cultures at 60 and 72 h treated with 5'-BrdU. We found average generation times of 31.57 h at 60 hrs and of 28.45 h, at 72 hrs, meanwhile in control was of 34.6 at 72 h. Apparently differences in cell cycle duration can be involved in Seckel syndrome.

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Chromosome 8p23 rearrangements. *G. Wenger[1,3], K. McBride[2,4], D. Bartholomew[2,4].* 1) Laboratory Medicine/Cytogenetics, Columbus Children's Hospital, Columbus, OH; 2) Division of Human and Molecular Genetics, Columbus Children's Hospital, Columbus, OH; 3) Dept. of Pathology, Ohio State University College of Medicine and Public Health, Columbus, OH; 4) Dept. of Pediatrics, Ohio State University College of Medicine and Public Health, Columbus, OH.

In recent years, repeated sequences located on the same chromosome have consistently been shown to be involved in the generation of recurring chromosome rearrangements including deletions, duplications, and inversions. Repeats located on non-homologous chromosomes are responsible for translocations including the constitutional t(11;22)(q23;q11.2) and t(4;8)(p16.1;p23.1). The olfactory receptor gene superfamily includes sequences located on almost all chromosomes, and has been implicated in the generation of recurring chromosome abnormalities including interstitial deletions, inv dup(8p), and a supernumerary marker chromosome. We describe a series of patients examined cytogenetically in our laboratory in the last five years, including two patients with interstitial del(8)(p21.3p21.3), three patients with duplication in the same region, a patient with duplication and deletion in the region, patients with the balanced translocation t(4;8) or resulting unbalanced karyotypes (two patients with der(8) and one patient with der(4)), and four patients with der(8) karyotypes as a result of inheritance of an unbalanced chromosome complement from a parental translocation in which the breakpoint for one translocation partner is 8p23.1. Olfactory receptor gene sequences have been found at the other translocation breakpoints. Clinical features included developmental delay and congenital heart disease. Studies in other labs have identified a maternal inversion polymorphism that favors the formation of rearrangements including inv dup(8p) and der(8p). Interstitial deletions and translocations may arise through this or other mechanisms also related to the presence of olfactory receptor gene sequences. Chromosome 8p23.1 rearrangements join the list of recurring microrearrangement syndromes as frequently encountered abnormalities in the clinical cytogenetics laboratory.

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A unique interstitial deletion involving chromosome 20q in a child with microcephaly and behavioral difficulties.

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Constitutional interstitial deletions involving the long arm of chromosome 20 are not common. To our knowledge, a deletion involving 20q13.11-q13.13 has never been reported. We describe a 7 year old boy with congenital microcephaly, dysmorphic features, and behavioral abnormalities due to a deletion involving chromosome 20q13.11-q13.13. This patient was born at term following an uncomplicated pregnancy to nonconsanguinous Chinese parents. He was healthy but noted to have microcephaly at birth. Hyperactivity and poor attention span were first noted at 4 years of age. Episodes of disruptive and aggressive behavior also developed, but there were no episodes suggestive of seizures. Early developmental milestones were normal, but at 7 years of age he had mild fine motor difficulties and learning difficulties, particularly in the area of speech and language. On examination, his head circumference plotted just below the 2nd percentile, his height was at the 25th percentile and his weight was at the 10th percentile. Dysmorphic facial features included hypotelorism, prominent nasal bridge, slightly flat philtrum, and micrognathia. Neurological examination was normal except for mild clumsiness of fine motor movements. Brain MRI was normal. Electroencephalogram at 4 years of age was normal. At 6 years of age the EEG showed spikes in the bilateral frontal, parietal, and temporal areas, particularly in sleep. At 7 years of age it showed frequent spikes in the left temporal areas. There were also frequent spikes in the left central parietal area; right central sharp waves; and mid-central temporal sharp waves. His karyotype revealed a small deletion within chromosomal region 20q13.11-q13.13. Parental karyotypes are pending. Further characterization of the interstitial deletion will be performed using a Sub-Megabase Resolution Tiling-set (SMRT) array-CGH (aCGH). Further characterization of our patients imbalance may allow a better understanding of our patients phenotype.

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PRENATAL DETECTION OF A PARTIAL MONOSOMY OF THE CHROMOSOME 13 AND PARTIAL TRISOMY OF THE CHROMOSOME 18 IN A FETUS WITH ALOBAR HOLOPROSENCEPHALY. *G. Razo-Aguilera, R. Báez-Reyes.* National Institute of Perinatology.

The holoprosencephaly (HPE) is a defect of the development of the previous brain and frecuently of the half face. It has a prevalence of 1:250 during the embriogenesis and 1:16,000 in newly born. It's associates with teratogen agents or genetic factors. The evidence for genetic causes of HPE for their heterogeneity comes of: 1) chromosomal anomalies, 2)family ocurrence and 3) genetic syndromes associates. The chromosomic aberrations are found in the 50% or more than the patients with HPE and exist already of 12 involved genes. The case of a couple of 35 years old both is presented, with 4 pregnancies, 1 alive son and 2 deaths with probably hidrocephaly, she is housewife and he is merchant, not consanguineous. They went to the consultation of Prenatal Diagnosis with 18 weeks of gestation for control and in the ultrasound of second level an unique ventricle was visualized in the brain, with adsence of thalamuses and the presence of a cardiopathy of the type CIV, the rest was normal. Genetic advice is given to the couple and we offered cytogenetic amniocentesis, they accepted and the result with G bands was reported: 46,XY,der(13)t(13;18)(q22;q23) desbalanced for a partial monosomy of the chromosome 13(q22-->qter) and partial trisomy of the chromosome 18(q23-->qter) of maternal origin, being carrier the lady of a balanced reciprocal translocation 46,XX,t(13;18)(q22;q23). The family study was completed. Most of the affected can to present alterations for desbalanced translocations; howewer, the reagment of this fetus is innusual, for what represents a special interest with regard to the segregation.

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Familial balanced homozygous translocation, t(11;22)(q23.3;q11.2) in a male - Segregation and recurrence risk - A case report. *D. Krishnamurthy[1], F.M. Mohammed[2], M.A. Redha[3], L. Bastaki[3], S.A. Al-Awadi[3].* 1) Cytogenetics and Molecular Cytogenetics, Canadian Specialist Hospital, Dubai, United Arab Emirates; 2) Allied Healtah Sciences, Kuwait University, Kuwait; 3) Kuwait Medical Genetics Centre, Maternity Hospital, Kuwait.

A balanced/unbalanced reciprocal translocation, t(11q22q) is one of the most common, familial site-specific reciprocal translocation which seems to have recurred over and over again, creating numerous carriers. In the majority of the reported cases ascertainment has been through the birth of a child with the chromosomal constitution 47,XX,+der(22) or 47,XY,+der(22), i.e., tertiary trisomy. Homozygosity for major structural rearrangement is very rare. A normal healthy, male and female, seeking premarital genetic counseling because of family history of mental retardation and chromosomal abnormality was investigated to exclude possibility of balanced chromosomal rearrangement. Metaphase chromosome analyses prepared from peripheral blood culture showed balanced homozygous translocation, 46, XY, t(11;22))(q23.3;q11.2), t(11;22))(q23.3;q11.2), Karyotype of the female partner was normal 46,XX. Balanced carriers are at risk of having offspring with the derivative 22 syndrome owing to 3:1 meiotic non-disjunction. Clinical features of the der(22) syndrome include mental retardation, craniofacial abnormalities and congenital heart defects. Heterozygous carriers pf t(11;22) are phenotypically normal, but are at high risk of having children with tertiaray trisomy 22 or recurrent fetal loss. Majority of the carriers reported in the literature are female. The family history is remarkable. The couple had three children (2 males and 1 female) 2 were balanced carriers, and 1 was affected with supernumerary chromosome, der(22). The recurrence risk and segregation of the reciprocal translocation will be presented.

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Cytogenetics of Idiopathic neurological conditions helped in identifying Glutamate and Gamma-amino butyric acid receptors as crucial genes in their etiology. *S. ponnala[1], V. Mohan[1], R.V. Alluri[1], S. Komandur[1], K. Chawda[2], K. Alladi[2], J.R. Chaudhuri[2], Y.R. Ahuja[4], Q. Hasan[2,3].* 1) Genetics and mol med, Kamineni Hospital, Hyd 500 068 India; 2) Dept of Neurology. Kamineni Hospitals, Hyd 500 068, India; 3) Dept of Genetics, BMMRC, Hyd 500 004, India; 4) Dept of Genetics, VMRC,Hyd 500 003, India.

Cases with learning disability, motor developmental delay with or without epilepsy pose diagnostic and therapeutic challenges to clinicians. In the present study idiopathic neurological cases were evaluated to define their genetic basis. 43 cases with delayed milestones, learning disorders with or without epilepsy were subjected to detailed neurological examination which included ENMG, EEG, CT scan and other relevant biochemical tests. cytogenetic evaluation was carried out. 27.9% patients showed chromosomal anomalies involving break points at 2q(2q23-24), 4q(4q21-26), 5q(5q31), 6q(6q21-24), 8p(8p22-pter), 8q(8q13-24), 15q(15q11-13) and 16q(16q22-24). Neurotransmitter receptor genes located at chromosomal break points were identified by OMIM search. 66.7% of the patients with chromosomal anomalies were associated with glutamate receptor and 33.3% with GABA receptor. L-Glutamate is the major excitatory neurotransmitter and its activation is responsible for basal excitatory synaptic transmission and plasticity, which is involved in cognitive functions. Disruption of the activity of this may be responsible for the clinical phenotype seen in 66.7% cases. While GABA receptor is a multi-subunit chloride channel receptor, which mediates inhibitory synapses in the brain and spinal cord. It plays a role in increasing or decreasing the cellular excitability by interfering with sodium, potassium and calcium influx, which may result in seizures or impaired neural functions. Altered function of GABA receptor may account for the phenotype seen in the remaining 33.3% cases. Loss or altered function of Glutamate and GABA receptors caused by chromosomal rearrangements appears to be a major factor for neurological dysfunction in the cases studied.

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Multiple spontaneous miscarriages caused by a rare and complex derivative chromosome resulting from reciprocal translocations involving chromosomes 4,7 and 15 in a phenotypically normal male. *J. Yung, S. Hardikar.* Cytogenetic laboratory, LabCorp/Dynagene, Houston, TX.

We are reporting a case of a couple that requested cytogenetic evaluation due to multiple miscarriages. The 26-year-old woman and 31-year-old man have had two previous first trimester spontaneous abortions. The woman also has a normal child from a previous marriage. The womans cytogenetic finding was 46,XX normal female. The mans chromosomal study revealed two reciprocal translocations. It was determined that the man carried two apparently balanced chromosome translocations, t(4;7)(q33;q22) and t(7;15)(p15;q24). These rearrangements resulted in a very complex derivative chromosome 7, der(7)t(4;7)(q33;q22)t(7;15)(p15;q24), consisting of chromosomal material from 4,7 and15. Given the complexity of the rearrangement, FISH and high-resolution studies were performed. The result confirmed the above break points. The presence of two translocations in one individual is rare. The two translocations involving one common chromosome, as in this case chromosome 7, is even more unusual. Patients with one reciprocal translocation are known to give rise to abnormal unbalanced offspring due to segregation at meiosis and meiotic crossing-over. An individual with such complex and unusual rearrangements will be unlikely to generate any offspring with a balanced karyotype and the incidence of unbalanced conceptus will be very high. Genetic counseling is warranted to discuss the reproductive consequences of carrying this complex chromosomal aberration and to address the recurrence risk for other family members who may carry a similar chromosome abnormality.

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Two infants with homozygous premature chromatid separation trait. *H. Numabe[1,2], T. Ikeuchi[3], T. Kajii[4], I. Kusakawa[5], I. Kato[5], S. Kosugi[1,2].* 1) Dept Clinical Genetics, Kyoto University Hospital, Sakyo-ku, Kyoto, Kyoto, Japan; 2) Department of Biomedical Ethics, Kyoto University Graduate School of Medicine, Kyoto, Japan; 3) Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; 4) Hachioji, Tokyo, Japan; 5) Department of Pediatrics, St. Lukes International Hospital, Tokyo, Japan.

We report two girls with homozygous premature chromatid separation (PCS) trait, chromosomally characterized by PCS and mosaic variegated aneuploidy (MVA). Case 1 had pre- and postnatal growth retardation, microcephaly, Dandy-Walker malformation, and minor facial abnormalities; and at age 22weeks developed generalized seizures controllable with phenobarbital. At age 4 months, bilateral multicystic Wilms tumors and rhabdomyosarcoma of the bladder were detected. She died of renal failure at age 9 months. Case 2 had pre- and postnatal growth retardation, malrotation of intestine, and duodenal stenosis caused by pancreas ring, but had not microcephaly nro Dandy-Walker malformation. At age 6 months, any tumor was not detected. All tissues and cells from the Case 1 analyzed showed a high frequency of PCS and MVA. On the other hand, blood cells from Case 2 and one of her parents analyzed showed a moderately high frequency of PCS, and MVA was observed only in Case 2. Case 2 and one of her parents had the same heterozygous mutation of *BUB1B* gene.

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Severe phenotype in a female with non-mosaic, non-ring X chromosome with Xp and Xq deletions and complete skewing of X-inactivation. *S.A. Vaz, J. Lee, S. Li, J.J. Mulvihill, S.E. Palmer.* Dept. of Pediatrics, Section of Genetics, Univ. of Oklahoma HSC, Oklahoma City, OK.

Females with X chromosome deletions commonly have a Turner syndrome-like phenotype. Severe phenotype in females with X chromosome rearrangements are uncommon, mostly due to small ring X chromosomes (usually mosaic), or rarely translocations or duplications; phenotypes include multiple anomalies and brain abnormalities. We report a female with Xp and Xq deletions and severe phenotype. She presented with dysmorphic features, hypertelorism, craniosynostosis, orbital asymmetry with mild unilateral microphthalmia, optic nerve hypoplasia, complete cutaneous 2-3 syndactyly of hand, and urogenital abnormalities. Intractable seizures developed during early infancy. Chromosome analysis, subtelomere FISH, and whole X chromosome paint showed 46,X,rea(X)(pterp22.33::p21q21::p22.33pter), with two copies of Xpter subtelomeres, one on each end of the rearranged X chromosome with Xqter subtelomere absent, confirmed with Xpter probe DSYS129. *KAL*, which maps 8 Mb proximal to DXYS129, was absent by FISH. The abnormal X was non-mosaic with de novo inheritance. Although this patient shares phenotypic features of X-linked single gene disorders which map to regions near the rearrangements, including Lenz (OMIM 301590) and oculofacialcardiodental (OMIM 300166) syndromes, anomalies resulting from disruptions of these genes seem less likely. Study of this patient using androgen receptor methylation showed X-inactivation to be 100% skewed. This patient shares phenotypic features with some cases of ring X chromosomes with non-random X-inactivation and severe phenotype presumed due to gene dosage effects. In these females, skewing usually occurs due to deleted *XIST*, which maps to Xq13.2; this band is proximal to the deleted region in this patient. However, several patients with ring X and retained but non-expressed *XIST* have been reported. Due to the presence of Xp subtelomere on both Xp and Xq arms as revealed by FISH, an etiology of initial complex deleted ring chromosome with subsequent subtelomere duplication and separation is suggested, with *XIST* most likely present but non-functional.

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Chromosome 10q deletion associated with diaphragmatic hernia. *L. Mehta[1], T. Vaughn[1], D.M. Barlev[2], D. Nedelea[1], J. Jacob[3], R. Kazi[3], A.L. Zaslav[3].* 1) Div Med Genetics,Schneider Children's Hospital at North Shore-NYU School of Medicine, Manhasset, NY; 2) Dept. of Radiology &; 3) Dept. of Laboratories, Long Island Jewish Medical Center, The Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY.

A 5 month-old male infant was evaluated for poor growth and dysmorphic features. Height, weight and head size were below 3rd % ile. Face was triangular with mild malar hypoplasia. Ears were lowset and overfolded. Premaxilla was prominent. There were bilateral single transverse creases on the palms and toes were overlapping. He had mild hypotonia and motor delays. He was hospitalized because of persistent mild respiratory distress. Chest X-ray showed a large, soft tissue mass in the right lower hemithorax with bowel loops occupying the right upper abdomen. CT scan confirmed anterior herniation of part of the liver into the right hemithorax, compressing the right side of the heart. Cardiology evaluation showed no structural heart defects. Standard cytogenetic analysis using G-banding revealed an abnormal karyotype of 46,XY,del(10)(q25). Parental chromosomes were normal indicating that the deletion was de novo. FISH analysis using 10p/q subtelomere probes (Vysis, Downers Grove, IL) confirmed the deletion in the patient and normal chromosomes in the parents. Phenotypic manifestations of chromosome 10q deletion include facial dysmorphisms, postnatal growth retardation, developmental and mental retardation, cardiac, and genitourinary defects. Our patient shows several of the described findings. To our knowledge this is the first case of del(10)(q25) presenting with a diaphragmatic hernia (DH). Most congenital DHs are posterolateral defects and left-sided. Anterior hernias (Morgagni), such as in our patient, are rare and typically right-sided. Most chromosome abnormalities associated with DH have been random. DH may occur in these cases as part of the generalized developmental disruption associated with genomic imbalance. This information will be of help in patient management and in defining the phenotype of chromosome 10q deletions.

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Features of DiGeorge/Velocardiofacial syndrome in a patient with chromosome 15q26.3 subtelomeric deletion. *K. Casas, L. Williamson-Kruse, J. Saunders, S. Li.* Dept Pediatrics, Univ Oklahoma HSC, Oklahoma City, OK.

A 7-year-old female presented with mild developmental delay, seizures, history of esophageal atresia and thymic hypoplasia at birth, and craniofacial features of the DiGeorge/Velocardiofacial syndrome (small head size, middle ear anomalies, prominent nose, and bifid uvula). Karyotype and fluorescent in situ hybridization (FISH) probe for chromosome 22q11.2 yielded normal results. Subtelomeric chromosome analysis revealed a chromosome 15q26.3 deletion. The patient was also found to have 11 pairs of ribs with partial fusion of right ribs 5 and 6, scoliosis, coalition of the carpal bones, and shortening of metatarsals 4 and 5. While several patients with more proximal deletions involving 15q26.2 have been reported with congenital diaphragmatic hernia and growth retardation, this may be the first report of a chromosome 15q26.3 deletion syndrome.

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Duplication 4p and autism: cytogenetic identification of a common region for autistic disorder. *J.J.D. Morrissette[1], L. Medne[3], R. McDaniell[3], A. Gupta[3], B. Parrish[2], C.E. Anderson[2], E.H. Zackai[3], N.B. Spinner[3].* 1) Div Path & Lab Med,; 2) Dept Pediatrics, St Chris Hosp for Children, Phila, PA; 3) Div of Human Genet, Childrens Hosp Phila, Phila, PA.

Autism is a behaviorally defined disorder believed to be heterogeneous. Autism has been found to run in some families, suggesting a genetic component to its etiology, and can be associated with genetic syndromes, and certain cytogenetic abnormalities. The most common cytogenetic abnormality observed is duplication of 15q11-q13, including the AS/PWS critical region, as well as several GABA-receptor genes. We identified three individuals with a diagnosis of Autistic Disorder based on DSM-IV criteria, who had chromosome abnormalities that resulted in partial duplication of 4p. Patient 1 is a 4 year old with a duplication of 4p14 to 4pter and a deletion of 8p21 [46,XX,der(8)t(4;8)(p14;p21)]. This individual had language and gross motor delays and had synophrys, cupped ears, high-arched palate, and self-injurious behavior. Patient 2 is a 21 year old who had a duplication of 4p12 to pter and a deletion of 4q28 [46,XY, der(4)t(4;4)(p12q28)]. This individual had bipolar disorder, cerebral palsy, hearing loss GE reflux, short stature, esotropia, and dysplastic finger and toenails. Patient 3 was a 17 month-old who had a duplication of 4p15 through 4pter [46,XX, dup(4)(p15)], with motor delays, prominent ears, delayed dentition, irregularily placed toes, with head circumference and length below the 5th centile. The smallest region of overlap includes 4p15 through 4pter. There are several genes of interest in this region, including SLIT2, a gene expressed in axons and growth cones during neural development, and dopamine receptor D5. A deletion involving 4q has been identified in another individual with PDD-NOS and a small, interstitial deletion comprising 4q31-q33. This deleted region contains genes that could be involved in the development of autism including a glutamate receptor, 2 glycine receptors and neuropeptide Y receptor. These 3 cases all share a region of overlap within 4p15 to pter, leading us to hypothesize that increased dosage of genes within this region leads to autism.

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Clinical diagnosis of human chromosome aberrations using a new technique combining DAPI staining and image analysis. *M. Ji[1], J. Liu[2], X. Wang[3], X. Ren[2], M. Liu[2], Q. Wang[2,4].* 1) The Laboratory of Human Geneti, Institute of Tumour of Zhongshan in Guangdong, Zhongshan, Guangdong, China; 2) Human Genome Research Center, Huazhong Univ of Sci and Tech, Wuhan, Hubei, China; 3) The Laboratory of Human Genetics, Institute of Planned Parenthood of Nanyang, Nanyang; 4) Center for Molecular Genetics, Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation.

In this study, human chromosome aberrations including several translocations, 46,XX,t(3;21)(12;18), 46,XX,t(11;22), 46,XY,t(7;22), and 46,XY,t(11;18) were identified by a new technique that combines DAPI staining and image analysis. The technique has been reported for analyzing plant chromosomes, but has not been applied to analyze human chromosomes yet. The staining method is conventional and the image analysis consists of only two steps: deconvolution and then three-dimensional reconstruction. The results showed that the karyotype of the 46,XX,t(3;21)(12;18) was 46,XX,t(3;21)(3q11.1;21p13), t(12;18)(12q15;18q23), and the karyotypes of the 46,XX,t(11;22), 46,XY,t(7;22) and 46,XY,t(11;18) were 46,XX,t(11;22)(11q23;22q13.1); 46,XY,t(7;22)(7q32;22q13.3) and 46,XY,t(11;18)(11q13.3;18q23), respectively. The derivative chromosomes involved in the translocations were also verified by chromosome painting as well as FISH with centromere probes. This new technique has two adavantages compared to the traditional G-banding technique: the results are more accurate and the procedure is more simple and convenient.

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Polymicrogyria in a child with deletion 9p24-pter and microduplication 22q11.2. *P. Callier[1], L. Faivre[2], N. Marle[1], C. Thauvin-Robinet[2], N. Madinier[3], A.L. Mosca[1], S. Ragot[4], F. Huet[3], J.R. Teyssier[4], F. Mugneret[1].* 1) Laboratoire de Cytogénétique,CHU Le Bocage, DIJON, France; 2) Génétique médicale,CHU Le Bocage, DIJON, France; 3) Pédiatrie,CHU Le Bocage, DIJON, France; 4) Laboratoie de Biologie Moléculaire, CHU Le Bocage, DIJON, France.

We report on a 9-year-old girl presenting with polymicrogyria and an unbalanced chromosomal rearrangement. She was the unique child of non consanguineous parents. Measurements at birth were normal. She was referred at 8-year of age for mental retardation with speech delay and seizures. At clinical examination, measurements were normal except progressive microcephaly (OFC -2.5 SD). Facial dysmorphism included synophrys, up-slanting palpebral fissures, low implanted hairs, large ears and hypotonic face. Cardiac and renal ultrasounds were normal, but cerebral MRI showed polymicrogyria, affecting preferentially the occipital and temporal region. Conventional cytogenetic analysis revealed a 46,XX,add(9)(p24pter). FISH studies with Vysis To TelVysion specific probes showed 9pter deletion. Array-CGH (GenoSensor Array 300, Vysis/Abbott, 287 DNA clones) confirmed the deletion 9pter from two clones (9pter and D9S913), but also showed that three clones (GSCL, HIRA and TBX1) were duplicated in the 22q11.2 region. Molecular Cytogenetics studies with Bacs located in the 9pter region are in progress to better define the size of the deletion. Although FISH studies on interphase cells with a HIRA 22q11.2 specific probe did not permit to confirm this microduplication, quantitative PCR (ABI Prism SDS 7700) using primers of the TBX1 gene region was in favour of an overexpression of the gene. Maternal cytogenetic studies were normal whereas paternal samples were not available. This study demonstrates the utility of the Array-CGH technology to detect complex rearrangements as well as microduplications that are not easily studied using FISH. This observation is also of interest since it could be a clue in the search of the genes responsible for polymicrogyria.

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Characterization by FISH of structurally abnormal Y chromosomes and of sex reversal cases: What are the implications on sex differentiation phenotypes? *M. Beaulieu Bergeron[1,4], M. DesGroseilliers[1,4], P. Brochu[3], E. Lemyre[2,4], N. Lemieux[1,3,4].* 1) Pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec, Canada; 2) Pédiatrie; 3) Pathologie et; 4) Centre de recherche de l'Hôpital Sainte-Justine, Montréal, Québec, Canada.

Infrastructural rearrangements of the human Y chromosome are often seen in both males and females. These structurally abnormal Y chromosomes are unstable and can be implicated in male infertility, azoospermia and sex reversal. When found in female patients, rearranged Y chromosomes can lead to gonadal dysgenesis, sex reversal, and increased risk of developing gonadal tumors (gonadoblastoma/dysgerminoma). We present the FISH results of 27 patients, including 16 cases of isodicentric Y chromosome (6 males, 9 females and one individual with sexual ambiguity), 5 cases of translocations between sex chromosomes (4 XX males and 1 XY female) and 6 sex reversed females with a normal Y chromosome. FISH analyses were performed using commercially available probes of both X and Y chromosomes, as well as BACs (Bacterial Artificial Chromosomes). Of the 16 patients with an idic(Y), all are SRY+, with the exception of a female bearing an idic(Yq) SRY-, which explains her phenotype. A predominant 45,X cell line in blood lymphocytes and/or gonads seems to explain the female phenotype of 6 other cases of idic(Y). As for t(X;Y) cases, a DAX duplication was found in the female patient, explaining her sexual differentiation despite the presence of SRY. In the remaining t(X;Y) cases, at least one of the XX males has breakpoints below the molecular region frequently rearranged in such cases. Finally, of the 6 females with a normal Y chromosome, one was diagnosed with probable familial androgen resistance, thus possibly explaining her condition. Also, gonadal mosaïcism was excluded in at least one case. These results therefore highlight the necessity of further molecular characterization in cases of structurally abnormal Y chromosomes and/or sex reversal, as this not only help explaining the phenotype of these patients, but could also improve genetic counselling and cares offered. *Support : RMGA-FRSQ and Fondation de lHôpital Sainte-Justine.*.

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Towards a molecular genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC) by applying microdissected sSMC probes on a 21.5 k BAC array-CGH. *A. Weise[1], K. Mrasek[1], N. Kosyakova[2], T. Liehr[1], P. Stankiewicz[3], S.W. Cheung[3], W.W. Cai[3].* 1) Institute of Human Genetics , Jena, Thuringia, Germany; 2) Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow 115478, Russia; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Small supernumerary marker chromosomes (sSMC) are present in 0.043% of newborns and about 2.5 million humans are carriers of sSMC. In about 30% of sSMC carriers, an abnormal phenotype is observed. Clinical outcome of sSMC is difficult to predict as various phenotypic consequences can appear due to (i) differences in euchromatic DNA-content, (ii) uniparental disomy (UPD) of the sSMCs homologous chromosomes, and/or (iii) different degrees of mosaicism. To address point (i) we microdissected DNA of 23 different sSMC cases by glass needle, amplified the obtained DNA by DOP-PCR and hybridized the product to a 21.5 k whole genome BAC CGH-array. To confirm the array data, the suggested breakpoint spanning BAC clones were applied in FISH on metaphase spreads from the three patients, who are presented here in more detail. The first case belongs to the most frequently observed sSMC group, an inv dup(15) case. The second case was an isochromosome 12p, found in patients with Pallister-Killian syndrome and the third one was an unusual sSMC derived from chromosome X, inherited from the patients father. We demonstrate that DOP-PCR amplified samples from the microdissected sSMC hybridized on CGH-array is straightforward in terms of time, use of resources, and sensitivity. Almost 50 years after the description of the first sSMC, molecular cytogenetics combined with array CGH provides a comprehensive approach for the characterization of sSMCs. The new knowledge coming from that should lead to an improved genetic counseling of cases with de novo sSMC based on a molecular genotype-phenotype correlation. Supported by the Dr. Robert Pfleger-Stiftung, BCM postdoc fellowship, Boehringer Ingelheim funds and DFG (436 RUS 17/109/04).

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One-hundred years of mental retardation and dysmorphic features solved: der(10)t(2;10)(q37;26)the phenotype described in 5 individuals over 3 generations. *R.L. Bennett[1], G.P. Jarvik[1], K.A. Leppig[2], C. Disteche[1].* 1) Div Medical Genetics, Univ Washington, Seattle, WA; 2) Group Health Cooperative, Seattle, WA.

The Medical Genetics Clinic at the University of Washington has followed the proband with MR and dysmorphic features since 1962. The 42 y.o. proband has a healthy older sister and two younger brothers, one of whom has a daughter with similar devleopmental delay and dysmorphic features. The proband's mother is healthy at age 66, and she had three similarly affected siblings, and two siblings with a more severe phenotype. The proband's maternal grandmother was unaffected, but she had a sister who died at age 21 and had a similar phenotype to the proband. Although the clinical and family history was compatible with a chromosome translocation, the chromosomal anomaly could not be identified by prometaphase G-banded cytogenetic analysis. Subtelomeric FISH analysis using the panel available from Vysis, Inc. identified an unbalanced .ish der(10)t(2;10)(q37;q26)(D2S447+,Z96139+,D102490+) result in the female proband and other affected family members. We review clinical data on affected relatives ranging in age from age 1 y. to age 71. Dysmorphic features include wide set eyes, broad and prominent nasal bridge, cupped ears, high palate, poorly aligned teeth, fifth finger clinodactyly, single palmar crease, short broad hands with shortened distal phalanges, IQ ranging from 60-70, overlapping of toes, flexion limitation at the elbows with limitations on supination and pronation, delayed motor and speech development, and seizure disorder. Two individuals within the family with a more severe phenotype with death during childhood likely inherited another unbalanced derivative chromosome. Subtelomeric FISH analyysis has permitted the identification of the etiology of approximately 6% of cases with MR of unknown cause as well as clarifying the basis of "private" family syndromes. It is for this reason that subtelomeric analysis has become a vital adjunct for routine cytogenetic analysis.

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Aneuploidy screening in mature human sperm of 40 infertile men before ICSI treatment. *G. Schwanitz[1], U. Paetzold[1], R. Schubert[1], K. van der Ven[2], M. Montag[2].* 1) Inst Human Genetics, Univ Bonn, Bonn, Germany; 2) Dep of Obstetrics and Gynecology, Univ Bonn.

The frequency of aneuploidy of chromosomes 8, 9, 11, 13, 15, 18, 21, X, Y and diploidy in sperm nuclei of 40 infertile probands and 10 healthy men of proven fertility were compared. The patient group was subdivided by the type of spermatogenic abnormalities into OAT, OT, AT and T comprising each 10 probands. Aneuploidy and diploidy frequency were analysed by FISH using repetitive and single copy DNA probes. The efficiency of hybridization was higher than 99% for each probe combination after development of specific hybridisation protocols. The total number of aneuploid nuclei with aberrations of the sex chromosomes was significantly increased in patients (0.33%) compared to the controls (0.18%; p.lt. 0.001). Of the 3 possible disomy combinations - XY, XX, and YY - the XY karyotype was more frequent in patients and controls with highly significant differences between the two groups (0.14, 0.08%; p.lt. 0.001). The total amount of aneuploidy of the autosomes was significantly increased in patients (0.45%) compared to the controls (0.26%; p.lt. 0.05). An unequal interchromosomal distribution of hyperploidy was found. The highest frequencies of disomy and nullisomy were observed for chromosome 11 in both patients (1.41 and 0.28%) and controls (0.80 and 0.15%). The chromosomes 8, 9, 13, 15, and 21 showed comparable amounts of disomic (0.14 - 0.18%) and nullisomic (0.07 - 0.11%) nuclei. Striking were the low aneuploidy rates of chromosome 18 in patients and control group (0.17, 0.08%). The disomy rates were higher than those of the nullisomies for all autosomes investigated. The frequency of diploid nuclei was significantly higher in patients (0.04%) than in controls (0.02%, p.lt. 0.001) but comparing them to aneuploidies the latter group was more than ten times higher for sex chromosomes and autosomes. There were differences among the four subgroups with OAT patients showing the highest and T patients the lowest rate of diploidy. The different types of spermatogenic abnormalities revealed a different quantitative and qualitative distribution of aberrations.

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A mosaic karyotype 47,XX,+r(Y)(p11.1?q11.2)/46,XX in a female patient referred to ICSI due to male factor infertility. *F. Dechend[1], B. Pabst[2], K. Miller[2], S. Schubert[2], G. Wilke[1], N. Graf[1].* 1) Humangenetik Hildesheim, GMP AJWG, Hildesheim, Hildesheim, Germany; 2) Institute of Human Genetics, Hannover Medical School, Hannover, Germany.

We report a case of a mosaic karyotype 47,XX,+r(Y)(p?11.1q11.2)/46,XX in a 27 year old female nullipara, who was cytogenetically diagnosed before performing ICSI. The woman is phenotypically and gynaecologically normal with the exception of an endometriosis uteri externa, but her 25 year old husband exhibits an OAT-syndrome. Chromosome analysis of lymphocytes from her subfertile husband revealed a normal 46,XY karyotype, while we found in about 60% of her lymphocyte cells a 46,XX karyotype and in 40% of her cells an additional ring chromosome Y. We were able to confirm these findings in fibroblasts from different tissues (ovar, skin and buccal mucosa). From FISH and PCR analysis it is suggested that the breakpoints of the ring chromosome Y are in p11.1, lacking most of the short arm, including SRY and the TSPY clusters; and in q11.2 between the AZFb and AZFc region. Although the TSPY clusters were deleted we could show by PCR and sequence analysis that at least one functional full-length TSPY copy is present. Whether the patient carries a risk for gonadoblastoma or has an increased risk for i.e. ovarian cancer is difficult to predict. To our knowledge, this is the first reported case of a chromosomal mosaicism with two X chromosomes and one ring Y chromosome. To elucidate the origin of the ring chromosome Y further studies are under progress.

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Paternally inherited interstitial duplication of chromosome 13q. *J.H.M. Knoll, D.L. Bridges, A.J. Miller, M.L. Begleiter, H.H. Ardinger.* Children's Mercy Hospitals and Clinics, School of Medicine, University of Missouri - Kansas City.

Inherited interstitial duplications of chromosomes are extremely rare. Four members of a family with an inherited duplication of chromosome 13q were studied. The proband was a 4 year old female with developmental delay and mild facial dysmorphology whose clinical findings overlapped those observed in chromosome 22q11.2 deletion syndromes. The region 22q11.2 was normal by FISH. Chromosome analysis with GTG-banding revealed a duplication of chromosome 13q22.1q32. A similar duplication was observed in the probands father and one of her older sisters. All carriers of the duplication had a history of developmental delay. One sister with normal intelligence did not carry the duplication. By FISH with a whole chromosome 13 paint probe, we confirmed the chromosome 13 origin of the additional chromatin on the abnormal chromosome 13. The duplication did not extend into 13q34 as FISH with a chromosome 13q subtelomeric probe (D13S327) showed a normal hybridization pattern. The duplication includes 3 chromosomal bands at the 550 band level and we estimate that it spans ~15 million nucleotides of DNA. Given the size of this duplication, it seems surprising that the phenotype is not more severe. Chromosome 13q22.1 q32 is being clinically dissected by examining in silico the genes in the region and comparing the phenotypic findings between duplication carriers.

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Craniosynostosis and distal 5q duplication. *J. Wang[1], M. Steinraths[1], B. Lomax[2], S.-L. Yong[2], M.I. Van Allen[2].* 1) Cytogenetics; 2) Medical Genetics, CW Health Centre of B.C., Vancouver, B.C.

Distal 5q duplication has been reported in less than 30 cases, with craniosynostosis present in six. We report two new cases with distal 5q duplication and craniosynostosis. The first case is a 6 month-old boy born at 39 weeks gestation by planned Caesarean section with low birth weight and length. He has an asymmetric skull and dysmorphic features including a long, flat philtrum and thin upper lip. Developmental delay is apparent at 6 months of age, primarily evident as gross motor delay. CT head at two weeks of age showed premature synostosis of sagittal, lamboidal and metopic sutures, with 3D reconstruction CT demonstrating multiple abnormal foramina. Cytogenetic and CGH analyses showed a karyotype of 46,XY,der(10)t(5;10)(q33.?2;q26.3). FISH analysis revealed both MSX2 and FGF4R genes to be duplicated. The second case is a 6 year-old girl born by emergency Caesarean section for failure to progress after spontaneous onset of labor at 39 weeks. Birth weight was 6 lb. 12 oz. (25-50th percentile). Prominence of the forehead and ridging of the metopic suture was noted from 5 months of age and a CT head demonstrated craniosynostosis of the metopic suture. Her height, weight and head circumference are all below the 5th percentile. She has facial dysmorphisms including bilateral epicanthal folds and ridging of the metopic suture. She has mild developmental delay, with notable delays in both expressive and receptive language. Bone age was advanced, with a bone age of 18-24 months at 14 months chronological age. Cytogenetic and CGH analyses showed a karyotype of 46,XX,der(17)t(5;17)(q35.1;p13.3). FISH analysis showed the Miller-Dieker critical region (17p13.3) to be intact. In consideration of our two cases and review of partial 5q duplication cases previously reported, including 7 cases with 5q31-qter, 6 cases with 5q32-qter, 5 cases with 5q34-qter, and 4 cases with 5q35.1-qter or 35.2-qter, we were able to refine genotype-phenotype correlations for distal 5q partial duplications. This will be of value in genetic counseling and clinical management of this previously diverse group of patients.

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Supernumerary derivative chromosome 15 in one of two developementally retarded twins - longitudinal study. *D. Hansmann[1], E. Tuschen-Hofstätter[2].* 1) Inst Prenatal Diagnosis, Meckenheim, Germany; 2) St. Marien Hospital, Children's Department, Bonn, Germany.

Indication for the present cytogenetic and clinical examinations was the developmental retardation of twins after in vitro fertilization following sterilisation. Since the maternal age at pregnancy was not increased, no invasive prenatal investigation was performed, particularly because 3 ultrasound scans carried out at gestational week 12, 16, 20 showed a normal development of both foetuses and no evidence of malformations. Twin II was delivered first by secondary caesarean section at gestational week 39. Apgar scores for both twins: 9 /10 after 5 /10 minutes. After an infection at the age of 4 weeks twin II suffered from acute asphyxia, lactacidosis, two cardiac arrests, and multiple organ failure. He developed a postinfectious hydrocephalus, infantile cerebral palsy, symptomatic epilepsy and severe developmental delay. Twin I at that time was slightly retarded in his developmental milestones. Except mild muscular hypotonia he showed no malformations or dysmorphisms. At the age of 12 months both children were severely retarded and chromosome analyses were performed on lymphocyte cultures. Twin II showed a normal male karyotype (46,XY, GTG, 30 mitoses, 400 B/G). Twin I revealed a supernumerary derivate 15 (SMC) in 30 mitoses, which was dicentric and contained euchromatic material between the 2 centromeres. Karyotype: 47, XY,+der(15)ish(pterq13::q13pter (GTG,CBG,WCP15,SNRPN). Chromosome findings in the parents were normal, thus the marker chromosome originated de novo in the patient. At the age of 5 years the boy showed the phenotype of 15q13 triplication. The question of whether in vitro fertilization leads to an increase in structural de novo aberrations is looked into on the basis of our own findings in 350 prenatal cases.

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Unusual case of double reciprocal translocation in a newborn. *O. Castro, K. Clark, G. Jervis, J. Moore.* Cytogenetics, Genzyme Genetics, Tampa, FL.

Cord blood from a newborn was studied for a maternally inherited t(1;16). Results showed a balanced t(1;16)(q12;q11.2)mat with an additional reciprocal translocation (rcp) involving the second chromosome 16 homologue, t(5;16)(p13;q24). FISH analysis showed these translocations to be apparently balanced. Chromosome analysis of the fathers blood showed the t(5;16)(p13;q24). Maternal testing had been performed previously (at another lab) due to a history of one healthy child and 4 spontaneous abortions (SAb). A search of Genzyme Genetics database from January 2002 through December 2004 showed 6 additional cases of double translocations (4 bloods and 2 amnios). Three cases (3/6) had 2 rcps; one case (1/6) involved the same homologue of chromosome 11 in both translocations. Three cases (3/6) had 1 rcp and 1 Robertsonian translocation (rob); two of the robs were unbalanced. Clinical indications were: Family history of translocation (4); Advanced maternal age (1); Failure to thrive (1) and Multiple congenital anomalies (1). Inheritance patterns of the 4 cases with 2 rcp (includes our original sample) showed that in 2 cases each parent contributed one translocation; these families also had repeat SAb. A third case had two maternal translocations following 2 abnormal amnios with 2 different translocations. The fourth case has unknown parental origin as maternal testing was normal and no paternal results are available. Two of the 3 rcp/rob cases showed paternal inheritance of the rcp with unbalanced de novo Robertsonian products. In the third case, the mother was a balanced carrier of both translocations, detected as the follow up to MCA in a child with the unbalanced rcp product. Clustering of translocations were seen with chromosome 11 (5 translocations); chromosome 13 (4 translocations); chromosome 16 (3 translocations) and chromosome 1 (3 translocations). Chromosomes 11 and 16 showed breaks in both arms; chromosome 1 and the acrocentric chromosomes only showed breaks in the long arm. Inheritance risk assessment may be available when each parent carries a translocation, but is extremely difficult to determine when an individual carries two translocations.

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Cytogenetic analysis on the placenta using primary cultures established from Percoll gradient purified trophoblast cells. *K.K. Ho[1], Y.M. Chin[2], W.S. Ng[2], J.A. Tan[1].* 1) Dept Molecular Medicine, Fac. Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Div of Genetics, Fac. Science, University of Malaya.

Cytogenetic analysis on term placentas is not commonly performed as compared to chorionic villi analysis mainly due to the inherently low success rate in culture setup of placental cells. The difficulties in establishing viable and proliferating primary trophoblast cultures has caused some laboratories to move from conventional G-banding analysis to molecular techniques such as FISH and CGH.

Percoll gradient centrifugation is the standard method to obtain highly purified and viable trophoblasts for establishment of primary cultures, which can be later used for biochemical assays and cell-matrix interaction studies. However, this utility has never been adopted for cytogenetic analysis as the primary trophoblast cultures used have never progressed beyond the first passage.

In this study, the potentials and pitfalls of utilizing Percoll gradient centrifugation to purify trophoblasts and to establish viable primary trophoblast cultures for placental cytogenetic analysis were evaluated. Trophoblast primary cultures were established according to a modified protocol of Kliman *et al.* (1986). In addition, cultures of explanted and digested villi from termed placentas were used as comparisons. Cultures were harvested using either the standard trypsin-dispersion method or cell cycle synchronization in order to obtain high-resolution chromosomes.

Cultures established from Percoll gradient purified trophoblast were free from tissue debris but exhibited spontaneous cell fusion, indicating differentiation of cytotrophoblasts into syncytiotrophoblasts. Occasionally, viable and proliferating cultures were obtained after prolonged culture for 3 weeks compared to 2 weeks for tissue explant cultures. Cell cycle synchronization was successfully performed using a MTX block followed by a thymidine release protocol. Chromosomes of 800 band level were attainable. Further work on the optimization of trophoblast cultures is still being carried out to ensure feasibility of routine cytogenetic diagnosis on term placentas.

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Developmental Genome Anatomy Project (DGAP): Characterization of Genes Critical to Specific Developmental Pathways. *D.J. Donovan[1], F.S. Alkuraya[1,2], G.A. Bruns[2,4], R. Eisenman[2], J.F. Gusella[3,4], D.J. Harris[2,4], A.W. Higgins[1,4], H.G. Kim[3,4], K.M. Kocher[4], S. Kulkarni[1], N.T. Leach[1,4], A.H. Ligon[1,4], W. Lu[1,4], R.L. Maas[1,4], S.D. Moore[1,4], F. Quintero-Rivera[3,4], I. Saadi[1,4], R.E. Williamson[4], B.J. Quade[1,4], C.C. Morton[1,4].* 1) Brigham & Women's Hospital, Boston, MA; 2) Children's Hospital, Boston, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Harvard Medical School, Boston, MA.

The Developmental Genome Anatomy Project (DGAP) is a collaborative effort to identify genes involved in human development and disease. By studying patients with apparently balanced *de novo* chromosomal rearrangements and at least one major congenital anomaly, we have correlated specific patient phenotypes to the disruption or dysregulation of genes at chromosomal breakpoints. From more than 180 cases, our progress to date includes over 130 FISH-mapped breakpoints from 64 cases. Of these, 80 breakpoints from 50 cases have been resolved to within a single BAC clone. Twenty-five breakpoints have been analyzed in detail and found to disrupt a gene directly. Attractive candidate genes for the autosomal form of Kallmann syndrome, seizures, behavioral disorders and hearing loss are among those recently identified. Eighteen breakpoints have been cloned, revealing nine microdeletions, one deletion, eight insertions, two duplications, and two balanced rearrangements at the nucleotide level. Most breakpoints fall within the intron of a gene, none fall within an exon, and one falls within a 3 UTR. Only three gene fusions have been found to date by RT-PCR. For the five instances in which the breakpoint fell outside of a gene, a gene appears to have been disrupted by the other breakpoint. Mutational analyses of individual collections with phenotypes similar to a DGAP case are ongoing in eight cases. We also are in the process of generating or characterizing mouse mutations in seven candidate genes from six DGAP cases. As the DGAP data collection has grown, we are working towards building a haplo-sufficiency map of the human genome for the developmental biology and medical genetic communities (http://dgap.harvard.edu).

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Congential hypothyroidism, atrial septal defect, and hearing loss associated with 7q31.3q32 interstitial deletion.
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We describe a young girl with a *de novo* interstitial deletion 7q31.3q32, and congenital hypothyroidism, congenital heart defect, and hearing loss. Pregnancy and delivery were unremarkable. Hypotonia and mild dysmorphism prompted cytogenetic studies in the newborn period. Routine G-banded chromosome analysis revealed a normal female karyotype. Congenital hypothyroidism was diagnosed following a positive newborn screen and thyroid replacement commenced at 6 days of age. An atrial septal defect was also diagnosed in the newborn period. In addition, the patient later developed bilateral sensorineural hearing loss. She has mild dysmorphic facial features. Expressive language is markedly impaired and there are behaviour concerns that include hyperactivity and aggressiveness. Growth parameters are within the normal range. At nine years of age she was re-evaluated and studies were undertaken to rule out subtelomeric anomalies. Reassessment of the karyotype identified a subtle interstitial deletion of the long arm of chromosome 7. Small deletions in this region of 7q are very rare in contrast to the larger deletions seen at the proximal and distal regions of 7q which are associated with recognizable phenotypes. The 7q31q32 region is known to contain genes that are associated with speech and language disorders/autism (FOXP2), and autosomal recessive deafness (DFNB4 and DFNB17). In addition, the gene for Pendred syndrome (SLC26A4) which is characterized by sensorineural hearing impairment, abnormal iodine transport in the thyroid gland, and temporal bone abnormalities, is located within this region. Monosomy for these genes could account in part for our patients phenotype. There are no genes in this region that have so far been implicated in cardiac development; however there are a number of reports of patients with congenital heart defects, particularly ASDs, who have deletions involving the 7q31 region. This area therefore may be of interest in identifying genes involved in cardiac development.

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Double paternal nondisjunction in an infant with transient neonatal diabetes mellitus and Klinefelter syndrome.
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Transient neonatal diabetes mellitus (TNDM) is a rare cause of severe hyperglycemia in infants with an incidence of approximately 1 in 400,000 live births. Insulin therapy is initially required with complete remission by 18 months of age. Approximately 40% of the patients developed diabetes mellitus type II later in life. TNDM is usually caused by over-expression of a paternally expressed imprinting locus on chromosome 6q24. This over-expression can be caused by paternal uniparental disomy (UPD), paternal duplication of the region 6q24 and loss of maternal methylation region at 6q24. Two genes, ZAC and HYMAI, located at 6q24, have relaxation of imprinting expression and are candidate genes for TNDM. We report on a baby who developed TNDM on the first day of life. He was born by cesarean section secondary to decreased fetal movements and poor fetal growth at 39 weeks of gestation. Birth weight was 1835 g and Apgar score 8 and 9 at the first and fifth minutes respectively. At day 1 he fed poorly and hyperglycemia with low levels of insulin was recorded. He was treated with intravenous insulin therapy with good resolution. At physical examination the baby was thin and macroglossia and hypertelorism were noticed. Karyotype showed 47,XXY chromosomes. DNA was collect from the baby and his mother. Microsatellite markers and the 50K XbaI GeneChip(Affymetrix) Mapping assay were analyzed. Both analyses showed no maternal contribution on chromosome 6. Using the 50K GeneChip, 405 out of 4100 markers on chromosome 6 were informative for UPD. The entire length of chromosome 6 showed no maternal contribution and 423 markers were heterozygous confirming paternal uniparental heterodisomy. The X chromosomes were one of maternal origin and the other of paternal origin. This suggests that both anomalies resulted from a double paternal meiosis I error, leading to paternal UPD6 and a sex chromosome anomaly. One previous case with XXX and UPD6 is also reported suggesting mechanisms predisposing to nondisjunction may be in play and that all cases of neonatal diabetes mellitus should be karyotyped.

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Higher zinc concentrations in seminal fluid are associated with lower levels of single and double strand DNA breaks in human sperm. *F. Marchetti[1], T.E. Schmid[1,2], R.H. Weldon[2], A. Baumgartner[3], D.W. Killilea[4], L.M. Montelius[4], B.N. Ames[4], D. Anderson[3], B. Eskenazi[2], A.J. Wyrobek[1].* 1) Biosciences Directorate, Lawrence Livermore Natl Lab, Livermore, CA, USA; 2) School of Public Health, University of California in Berkeley, Berkeley, CA, USA; 3) Department of Biomedical Sciences, University of Bradford, Bradford, UK; 4) Childrens Hospital Oakland Research Institute, Oakland, CA, USA.

Zinc has essential roles in a wide range of biochemical and cellular processes, and zinc deficiencies have been associated with both chromosomal damage and infertility. Zinc is especially critical in sperm development, where it is predominantly located in the outer dense fibers of sperm flagella and in the nucleus where it binds stoichiometrically with protamine 2 during compaction of sperm chromatin. We examined the association between the levels of zinc in seminal plasma and the magnitude of sperm DNA strand damage in a cohort of 87 generally healthy non-smoking male volunteers with no history of fertility problems. Single sperm electrophoresis (Comet assay) under alkaline and neutral conditions was employed to measure single-strand DNA damage (breakage or alkali-labile sites) and double-strand DNA breakage, respectively. Zinc seminal fluid levels were determined using inductively coupled plasma-absorption emission spectroscopy (ICP-AES). Seminal zinc concentration was inversely associated with the magnitude of single- and double-stranded DNA damage, i.e., percent DNA in Comet tail under alkaline (=-0.018, p=0.008) and neutral conditions (=-0.026, p=0.003) after adjusting for age, duration of abstinence, and daily caloric intake. These findings suggest that men with lower levels of zinc in their seminal plasma carry more DNA breaks in their sperm. DNA breaks in sperm are expected to increase the risk for heritable chromosomal aberrations in the offspring, which would be expected to increase their risks for malformations and other developmental defects. This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory, contract W-7405-ENG-48, with funding from NIEHS Superfund P42ES04705.

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Vitamin C intake modifies the effects of aging on DNA strand damage in sperm. *T.E. Schmid[1,2], B. Eskenazi[2], F. Marchetti[1], S. Young[2], R.H. Weldon[2], A. Baumgartner[3], D. Anderson[3], A.J. Wyrobek[1].* 1) Biosciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; 2) School of Public Health, University of California in Berkeley, Berkeley, CA; 3) Department of Biomedical Sciences, University of Bradford, Bradford, UK.

The trend for men to have children at older ages raises concerns that age may increase the risk of transmitting germ-line mutations that will be detrimental for the genetic health of offspring. Lifestyle factors such as diet may reduce such paternally transmitted genetic risks. In particular, vitamin C (ascorbic acid), a known scavenger of reactive oxygen species (ROS), has been shown in prior studies to reduce oxidative stress in sperm in vitro. We hypothesized that advancing age induces DNA strand damage in sperm and that vitamin C intake reduces the impact of aging on sperm DNA damage. We used sperm electrophoresis to detect single-strand DNA damage (alkaline sperm Comet assay) in the sperm of 90 non-smoking men (range 22-80 years) with no known fertility problems. We found that the amount of DNA damage (i.e., percent sperm DNA in the comet tail) was higher in the 60-80 than in the 20-29 year age groups (50 vs. 43%; p=0.03); adjusting for covariates, there was a 0.2% increase in sperm DNA damage per year of age (p=0.003). Independent of age, men with high daily vitamin C intake had significantly less sperm DNA damage than men with low intake (38 vs. 45%; p=0.006). When we dichotomized the intake of vitamin C at the median, we observed an increase in DNA damage with increasing age among low consumers of vitamin C (p=0.004), but no increase with age in those who consumed higher levels of vitamin C (p-value on the interaction term of age and vitamin C intake = 0.03). These findings suggest that a component of sperm DNA damage seen with age is the consequence of increased oxidative stress in the male reproductive tract. [This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory, contract W-7405-ENG-48, with funding from NIEHS Superfund P42ES04705 and European Community contract QLG4-CT-2002-51611.].

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A neocentromere derived from a supernumerary marker deleted from the long arm of chromosome 6. *N. Qin[1], J. Bartley[1], J. Wang[2], J. Jogenson[1], P.E. Warburton[3].* 1) Genzyme Genetics, Orange, CA; 2) Genzyme Genetics, Pasadena, CA; 3) Mount Sinai School of Medicine, New York, NY.

Neocentromeres are new centromeres formed at previously non-centromeric locations. A neocentromere is a rare phenomenon; to date, 72 cases of neocentromeres involving 19 different human chromosomes have been described [1, 2, 3]. Chromosomes involved include X, Y, 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, and 21. It has been assumed that the neocentromere can occur on any of the chromosomes. In the present study, we report a first case of a neocentromere derived from chromosome 6. Cytogenetic studies were performed on the parents of a phenotypically abnormal child with a balanced reciprocal translocation between chromosomes 4 and 15 (this test performed by another lab). The mother had normal karyotype. However, though phenotypically normal, the father had the same 4;15 translocation as his child. In addition, he had an interstitial deletion of the long arm of chromosome 6 between bands 6q16.2 to 6q22.2, as well as a supernumerary marker chromosome in every cell examined. FISH studies indicated that the marker was derived from chromosome 6 and contained no alpha satellite and no subtelomere sequences. Immunofluorescence analysis showed a positive signal for the CENP-C antibody, confirming the neocentromere formation. Review of the childs karyotype showed, in addition to the 4;15 translocation, an interstitial deletion in the long arm of chromosome 6 with no marker chromosome. Therefore, the abnormal phenotype of the child arises from the interstitial deletion, and not the translocation. Our case adds chromosome 6 to the growing list of chromosomes for which neocentromeres have been reported. In this presentation, we will also delineate the clinical phenotype associated with an interstitial deletion of 6q16 to 6q22.

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A balanced de novo translocation t(15;16)(p11.2;q11.2) mediates a position effect in an Angelman-like patient. *P. Finelli[1,2], S. Russo[1], M. Masciadri[1], M.P. Recalcati[1], D. Giardino[1], F. Faravelli[3], L. Larizza[4].* 1) Lab Medical Genetics, Istituto Auxologico Italiano, Milan, Italy; 2) Dip. di Biologia e Genetica Università di Milano; 3) Struttura Complessa Genetica Umana, Ospedali Galliera, Genova; 4) Genetica Medica, Dip. di Medicina, Chirurgia e Odontoiatria, Università di Milano.

We report on a patient referred to our laboratory as affected with Angelman Syndrome. The more common known defects underlyng AS, as 15q11-q13 microdeletions, 15qpatUPD and IC microdeletions were ruled out. Cytogenetic analysis revealed the presence of a de novo balanced translocation t(15;16)(p11.2;q11.2). FISH characterization, by using -satellite and DNA/rRNA coding sequences allowed to locate the 15p bkp in 15p11.2, within heterochromatic sequences, while BACss contig (16q11.2), allowed to map the 16q bkp in the CDA08 gene interrupting it. Quantitative analysis performed by Real Time PCR showed the presence of a truncated transcript lacking the COOH-coding portion of the gene, confirming the previously identified bkp mapped between exons 11-12. As the 15 bkp is within heterochromatic region, we focused on 16q bkp region, selected genes flanking the bkp and investigated whether their expression profile and function might be related to the patients phenotype. Preliminary results of the in progress quantitative analysis of different gene transcripts, by Real Time PCR, evidenced a halved expression of the NETO2 gene, not disrupt, located at 11 Kb centromerically to CDA8. We aim at studyng other flanking genes to verify whether the patient's severe neurological phenotype might result from structural haploinsuffinciency of CDA8 and functional haploinsufficiency of NETO2 and additional genes. The latter effect may be mediated by the separation of cis-acting elements in 16p11.2 or by the local alteration of chromatin structure. Although it's currently unknown which mechanism disturbs the NETO2 transcriptional control, a further example of overthrow of gene expression through position effect caused by chromosomal rearrangement is delineated with potential disclosure of novel genes not involved in genetic diseases.

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Study of meiotic segregation in three men with 14;22 Robertsonian translocation. *K. MORADKHANI[1,2], J. PUECHBERTY[1,2], S. BHATT[1,2], G. LEFORT[2], P. VAGO[3], P. SARDA[2], F. PELLESTOR[1].* 1) Cytogenetics of Human Gametes, Institut of Human Genetics, Montpellier, Languedoc, France; 2) Department of Medical Genetics, Arnaud de Villeneuve University Hospital, Montpellier, France; 3) Department of Cytogenetics, CHU Clermont-Ferrand, France.

Study of meiotic products by fluorescence in situ hybridization (FISH) in male carriers of Robertsonian translocations constitute an efficient tool for estimating the proportion of normal sperm (alternate segregation) in male patients with chromosome abnormalities. The information derived can be used as a key element in genetic counseling and decision making in the choice of assisted medical procreation (AMP) methods. To date, the meiotic segregation of rob(14q22q) has not been studied. We present the results of sperm analysis of three men heterozygous for 14;22 Robertsonian translocation. The three translocation carriers, with (1) oligoasthenoteratospermia, (2) teratospermia and (3) normospermia, were ascertained through fertility work-up. After fixation on slides, sperm nuclei were decondensed using 0.5M NaOH solution. Sperm analysis was performed by dual color FISH technique, using either directly labelled locus-specific probes (LSP) or whole chromosome painting probes (WCP). A mean of 5250 sperm nuclei were scored per patient by Locus specific probes (ranging from 5087, 5237 and 5428 respectively) and 4704 sperm nuclei per patient by WCP (3279, 5330 and 5504 respectively). The mean frequencies of normal and balanced spermatozoa ranged from 78.97% for LSP and 81.01% for WCP. When compared, both LSP and WCP probes gave similar results for segregation patterns, demonstrating the efficiency of the two procedures for sperm study in Robertsonian translocation carriers. This study shows that the segregation pattern of this rare Robertsonian translocation is identical to that of the rare and common Robertsonian translocations previously analysed. These data support the existence of similar meiotic behaviour for all the nonhomologous Robertsonian translocations. This study was supported by a French reseach project PHRC (N7732) from the C.H.U. of Montpellier.

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Male intertility,45,X/46,X,del(Y)(q11.22)/46,XY mosaic karyotype, and Y chromosome microdeletions in a 42 year-old man. *L. Fan[1], R. Fetni[2], R. Mio[1], A. Chan[1], P. Scott[1].* 1) Montreal Children's Hospital, McGill University Health Center, 4060 ST-Catherine W, PT-420, Montreal, Quebec, Canada; 2) St-Justine Hospital, University of Montreal, 3175 Cote-St-Catherine, Montreal, Quebec,Canada.

We present the results of laboratory investigations, with emphasis on the Y chromosome, in a male referred for infertility. Cytogenetic analysis revealed the presence a mosaic karyotype of the following constitution: 45,X (50%); 46,XY (12.5%); 46,X,del(Y)(q11.22) (37.5%). Molecular analysis using PCR amplification of the AZF regions and band densitometry revealed partial deletion of the AZF regions -b and -c, consistent with cytogenetic findings. Unique to this patient is the observation of a normal cell line and a rearranged Y chromosome in the form of a deletion of the long arm following cytogenetic and molecular analysis. These findings support the use of PCR-based analysis of Y chromosome organization, in addition to cytogenetic analysis, in the diagnosis and clinical management of male infertility, even in the presence of mosaicism.

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Characterization of a Xp11.22 microdeletion in a male patient presenting with failure to thrive, mental retardation, dysmorphic features, microgenitalia and tubulopathy of Dent's type. *V. Drouin-Garraud[1], F. Broux[2], P. Saugier-Veber[1,3], G. Joly-Helas[4], E. Bessenay[1], T. Frebourg[1,3].* 1) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Department of Pediatric, Rouen University Hospital, 76031 Rouen, France; 3) Inserm U614, Faculty of Medicine, 22 boulevard Gambetta, 76183 Rouen, France; 4) Laboratory of Cytogenetics, Rouen University Hospital, 76031 Rouen, France.

Dent's disease (DD) is a rare X-linked hypercalciuric nephrolithiasis resulting from inactivating mutations within the *CLCN5* gene located in Xp11.22 and which encodes a voltage-gated chloride channel. DD is characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, rickets and eventual renal failure. F. is a 13 years old boy born at term after an uneventful pregnancy with normal mensurations. He presented at birth with dysmorphic facial features and high vaulted narrow palate, hypogenitalism, ventricular septal defect and horseshoe kidney. He developed a tubulopathy with hypercalciuria and nephrocalcinosis at the age of seven associated with mental retardation and failure to thrive. Ophthalmologic examination was normal. At 13 years, he had no language, very short stature (1,15m, -6 SD) and dysmorphic facial features. His mother and one of his two sisters had obvious dysmorphic facial features. R-banding lymphocytes chromosomal analysis (550 bands level) was normal. The existence of a tubulopathy compatible with the diagnosis of DD in this boy in association with numerous unrelated symptoms led us to suspect a Xp11.22 microdeletion involving the *CLCN5* gene. Indeed, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments), we identified a microdeletion removing the entire *CLCN5* gene and spanning on at least 2 Mb. His mother and his two sisters also carried the microdeletion. This case report represents the first case of a DD associated with developmental and malformative syndrome in the context of a Xp11.22 microdeletion. Fine mapping of this Xp11.22 microdeletion would facilitate the positional cloning of a gene involved in X-linked mental retardation.

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Unusual clinical features observed in a young girl having an inherited maternal translocation (13;14) with a mixed maternal uniparental hetero and isodisomy 14. *K. Krabchi[1], M. Ferland[1], F. Halal[2], L. Russel[2], A.M.V. Duncan[3], R. Drouin[1].* 1) Dept of Pediatrics & Service of Genetics, Sherbrooke Univ, Qc, Canada; 2) Div of Medical Genetics, McGill Univ Health Ctre, Mtl, Qc, Canada; 3) Lab of Cytogenetics, Montreal Childrens Hosp, Mtl, Qc, Canada.

We report a case of a 20-month-old girl who has inherited maternal balanced Robertsonian translocation detected by conventional cytogenetic techniques. Her phenotype included : growth retardation, hypotonia, significant feeding difficulties, hyperextensible joints, microcephaly, small anterior fontanel, prominent forehead, telecanthus and depressed nasal bridge. The probands clinical and cytogenetic features alerted us about the possibility of an uniparental disomy (UPD). By karyotyping her asymptomatic mother and sister, we identified in both the same balanced translocation. Concurrently, we excluded the possibility of a trisomy 14 mosaicism. In order to demonstrate UPD and to ascertain the parental origin of both chromosome 14, a molecular genetic study using microsatellite markers specific to chromosome 14 was performed. The distribution of the markers on the chromosome 14 suggested the presence of a heterodisomy of the proximal and distal long arm segments combined to an isodisomy of the median long arm segment. This UPD14 was of maternal origin and most likely resulted from a double meiotic chiasma between chromosome 14 homologues. The analysis using highly polymorphic markers specific for different chromosomes has confirmed the biparental origin of chromosomes 13 and excluded a false-paternity. Moreover, the sister presenting the rob t(13;14) has inherited the derivative 13;14 from her mother and the free chromosome 14 from her father. This particular form of UPD14 could be the consequence of a chromosome nondisjunction at the maternal meiotic stage I division which was followed by the loss of a chromosome 14 from a trisomic zygote. Furthermore, an imprinting effect resulting from the isodisomy segment of matUPD14 could possibly explain the bilateral nephrocalcinosis, the hypercalciuria and the myopathic electromyogram, three additional features observed in the proband and not previously reported in matUPD14.

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Molecular cytogenetic study of a 45,X male with cryptic Y/15 translocation and deletion of IGF1R gene. *D. Mercer[1], C.T. Dvorak[1], D.L. Wesley[1], C. Techakittiroj[1], H.C. Andersson[1, 2], T. Dise[2], M.M. Li[1, 2].* 1) Human Genetics Program; 2) Dept Pediatrics, Tulane Univ Med Sch, New Orleans, LA.

A male newborn prenatally diagnosed with Turner Syndrome was referred for genetic evaluation due to the apparent phenotype/genotype discrepancy. He was born at term to a G2P1 28-year-old mother with normal male genitalia and bilateral descended testes. Physical examination revealed that his height, weight, and head circumference data were well below the 5th percentile. He also showed some minor dysmorphic features including mild midface hypoplasia, bilateral epicanthal folds, a depressed nasal bridge, bilateral preauricular pits, small year pits, and mildly puffy feet. No cardiac problem was observed. High-resolution chromosome analysis demonstrated a karyotype of 45,X. FISH with DNA probe for the SRY gene showed a positive signal on the telomere region of one chromosome 15q, which was negative for the 15q telomere probe and positive for the Xp/Yp telomere probe. FISH with the IGF1R probe (BAC clone RP11-262P8) demonstrated a deletion on one chromosome 15. Severe growth retardation was observed during follow-ups. At 4 months of age, his head circumference was 35cm (-4 SD), his height was 52cm (-3.66 SD), and his weight was 4.5kg (-2.2 SD). Cytogenetic study on the mother of the patient showed a normal female karyotype. The father was not available for study; however, two half-sisters from the same father were phenotypically normal. 45,X male has been rarely described in the literature. The chromosome rearrangements in these cases have usually involved translocation of all or part of Yp to an autosome. Most of these patients display sexual development delay and azoospermia due to the loss of some Y material. Additional manifestations vary significantly depending on the autosome involved. The growth retardation and mild dysmorphic facial features in our patient are consistent with the phenotype of patients with the deletion of 15q26.3. Although 100% of Turner patients display short stature, more than 50% of 45,X males are of normal height. Therefore, the severe growth retardation in our patient is at least partially resulted from the deletion of one copy of the IGF1R gene.

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Autosomal imbalance enhances apoptosis during neuronal differentiation of mouse ES cells. *Y. KAI[1], Y. KAZUKI[1], S. ABE[1], M. TAKIGUCHI[1], S. KISHIGAMI[2], T. WAKAYAMA[2], C.C. WANG[3], M. OSHIMURA[1].* 1) Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, Tottori University, Japan; 2) Laboratory for genomic Reprogramming Center for Developmental Biology, RIKEN, Japan; 3) Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Shatin, Hong Kong.

Most individuals with autosomal imbalance such as aneuploidy are associated with mental retardation, though the particular chromosomal syndromes are phenotypically and/or clinically distinct. In the case of Down syndrome (DS), the most common autosomal aneuploidy, a reduction in both the number and density of neurones in the brain is a common abnormal phenotype. Recently, we established an in vitro model system for neuronal differentiation using mouse ES cells to examine the early stage of neurogenesis. A model of DS was created by introduction of an extra human chromosome 21 into the ES cells [ES (hChr.21)]. Interestingly, we observed a higher incidence of apoptosis during the in vitro early neuronal differentiation in the ES(hChr.21) cells. Here, we ask if chromosomal imbalance commonly induces higher levels of the apoptosis. Toward that end, we isolated mouse ES cells with an introduced human chromosome 21, 11 and 6p, and with an additional mouse chromosome such as chromosome 1, 8, and/or 17. All the clones showed an elevated incidence of apoptosis during the in vitro neuronal differentiation. Loss of mouse Y chromosome, i.e., missing sex chromosome, does not have the effect. Our findings strongly support the hypothesis that autosomal imbalance is commonly associated with reduced number of neuronal cells. The reduction in the number of neuronal cells may result in mental retardation in patients with chromosomal aneuploidy syndrome. Our data further suggest that a common molecular event is present for recognition of autosomal imbalance and the consequence.

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Metabolic decompensation in two unrelated children with small autosomal aberrations. *S. Wu, S. Yano, R. Boles.* Dept Pediatrics, Children's Hosp, Los Angeles, Los Angeles, CA.

Episodes of clinical deterioration associated with intercurrent illness and/or decreased caloric intake suggest the presence of a possible inborn error of mitochondrial metabolism, especially if associated with vomiting, altered mental status, metabolic acidosis or a rapid response to sugar intake.Potential diagnoses include the organic acidemia, fatty acid oxidation disorders and mitochondrial cytopathies. We present two infants with small chromosomal deletions, in whom intercurrent illness was associated with altered mental status and/or vomiting and metabolic acidosis, which resolved rapidly upon administration of sugar-containing fluids. The first Hispanic female presented at birth with cholelithiasis, arthrogryposis, severe symmetrical growth retardation and microcephaly. The patient has a karyotype of 46,XX, der(4)t(4;8)(p16.1;p23.1) with monosomy 4p16.1- pter and trisomy 8p23.1- pter. The second Hispanic female presented at age 3 months with dehydration, acidosis, growth retardation, down-slanting palpebral fissures, high arched palate, ezcema, lumbar kyphosis, hypercalcemia and significant lymphedema of the extremities. Peripheral blood karyotype revealed a small interstitial deletion between the bands 3q13.1 and 3q21. These infants may be predisposed towards metabolic decompensation under stress, possibly due to haploinsufficiency of one or more proteins involved in energy metabolism. Therefore, we recommend that individuals suspected to have mitochondrial disease, especially if associated with dysmorphic features or malformations, should be screened for potential chromosomal abnormalities.

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Characterization of high-resolution G-banded chromosomes - Enhancement of banding pattern using different cell synchronization agents. *W.S Ng[1], K.K. Ho[2], Y.M. Chin[1], J.A. Tan[2].* 1) Div Genetics, Fac Science, University of Malaya, Malaysia; 2) Dept Molecular Medicine, Fac Medicine, University of Malaya, Kuala Lumpur, Malaysia.

G-banding remains the gold standard in clinical cytogenetics despite the introduction of new molecular techniques such as FISH, and CGH. Continuous development and research in banding protocols have lead to G-banding of high-resolution chromosomes with the application of special staining to enhance specific banding patterns. High-resolution chromosomes are obtained through cell-cycle synchronization and are useful to visualize minute aberrations on the chromosomes.

Several cell cycle synchronizing agents in various combinations were evaluated on trophoblast cell cultures, and specific agents were observed to have characteristic effects on the G-banding patterns. The chemicals evaluated were MTX, BrdU, FrdU, thymidine and uridine. Length of each treatment followed closely the timings used with high-resolution blood cultures. The banding patterns produced were compared and characterized.

BrdU was observed as the superior releasing agent for either MTX or FrdU/Ur blocked cell cultures. Higher percentage of high-resolution chromosome spreads of 800 band level was harvested using this treatment. The dark bands in the chromosomes were more intense and distinct when the Wrights stain was used. Sub-bands were observed as a result of further resolution of the minor bands, for example, the distal arm of chromosome 1p which is usually poorly stained was consistently banded with Wrights stain. Although colchicine treatment promotes chromosomal condensation, addition of colchicine 3 hours prior to harvesting did not affect chromosome length, but increased the mitotic index.

Staining enhancements observed in BrdU incorporated chromosomes may be due to interference of this nucleotide with normal chromosomal condensation as well as favorable interaction with Wrights stain. Therefore 2 advantages with cell cycle synchronization; high-resolution chromosomes as well as enhancing the G-bands.

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A frequent partial AZFc deletion does not render an increased risk of spermatogenic impairment in East Asians.

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The gene families in the AZFc region of the Y chromosome have been shown to be functionally important in human spermatogenesis. The gr/gr deletion, a partial AZFc deletion that reduced the copy numbers of all the AZFc gene families, was identified as a significant risk factor for spermatogenic impairment in Dutch, Spanish and Italian. However, the presence of this deletion in the healthy French and German questioned its importance in male infertility. In this study, we showed that the gr/gr deletion did not render an increased risk in Han Chinese. In fact, the gr/gr deletion is frequent (~8%) in our survey of 886 East Asians from 8 ethnic groups. Furthermore, the DAZ1/DAZ2 deletion was detected as the primary subtype of the gr/gr deletion in East Asians, though this doublet was considered to be crucial for normal spermatogenesis in Europeans. Our observations suggested that the functional difference between the AZFc gene copies was a likely cause of inconsistent associations of the gr/gr deletion with spermatogenic impairment across populations.

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Pattern of human satellites on chromosome Yq12. *M. Babcock[1], S.A. Yatsenko[2], J.R. Lupski[2], P. Stankiewicz[2], B. Morrow[1].* 1) Albert Einstein Col Medicine, Bronx, NY; 2) Baylor College of Medicine, Houston, TX.

Satellite DNAs from the heterochromatic regions of the human genome comprise a class of tandemly repeated non-coding sequences that were originally identified by density gradient centrifugation. The breakpoints of the recurrent constitutional t(11;22) translocation cluster in satellite sequences within the LCR22-3a low copy repeat block on chromosome 22q11.2. Additional satellite sequences map in other LCR22s and they may be involved in chromosome rearrangements. We hypothesized that they could confer chromosome instability elsewhere in the genome. To determine their copy number, we performed Southern blot hybridization on human genomic DNA digested with different restriction enzymes. Genomic Southern blot analysis on SpeI digested human DNA using a satellite I probe revealed two fragments (5 and 2.4 kb) that were present in a very high-copy number. The 2.4 kb fragment was previously reported to be enriched in male DNA and was termed HSATI (PMID, 6324132). We performed FISH mapping with an HSATI probe and found that it painted the entire heterochromatin of the long arm of chromosome Y in human, Yq12. Interphase FISH mapping showed multiple hybridization signals on the periphery of the nucleus, a location where heterochromatic regions are known to reside. The structure of chromosome Y is highly variable in evolution, even among hominoid species. FISH mapping with human HSATI was performed on chimpanzee, bonobo, gorilla and orangutan cell lines and showed no fluorescence signal, indicating that it is specific to human chromosome Y. The Yq12 region also contains a pentamer repeat, (CATTC)n, referred to as HSATII. To determine the co-localization pattern of HSATI and HSATII on Yq12, we performed metaphase, interphase, and fiber FISH mapping. We found an interspersed pattern of these two satellite DNAs spanning Yq12, with HSATII being the predominant species. Taking classic molecular biological approaches, we have determined the pattern of interspersed satellites on human Yq12 that are missing from the human genome sequence.

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Asynchronous replication of biallelically and monoallelically expressed loci in triploidy. *O. Reish, D. Cooltin, M. Mashevich.* Genetic Inst, Assaf Harofeh Medical Ctr, Zerifin, Israel.

Purpose: Triploidy results in vast majority of cases in fetal loss and in few cases in multiple anomalies with perinatal death. The secondary genetic effects resulting from the extra haploid chromosomal set havent been evaluated yet. Here the level of synchronization in replication timing of alleles and genomic instability associated with other anuepolidies were assessed in triploid cells. **Methods:** Fluorescence in situ hybridization was used for 4 loci in amniotic cells or lymphocytes derived from 4 trisomy 21 cases, 5 controls and 5 triploidies. **Results:** Biallelically expressed genes (21q, RB1 and c-myc), showed a significantly higher level of asynchronous replication in the trisomy 21, and triploid cells comparable to control group. Genomic instability as demonstrated by secondary aneuploidy, was significantly increased in both trisomic and triploid cells comparable to the control. Monoallelically expressed gene (SNRPN), showed an altered replication pattern in triploid cells, however, no alteration was detected in trisomy and control cells. **Conclusions:** Altered replication timing of biallelically expressed genes and secondary genomic instability were detected both in trisomic and triploid cells. However, alteration of monoallelically expressed genes that was detected in tripolid cells but not in trisomy cells may suggest a possible cellular replication control hierarchy where imprinted gene get affected only when severe cellular insult such as the presence of an extra haploid chromosomal set occur.

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Identification of a specific interstitial telomere-like sequence (ITS) in human chromosome 22 at q11.2. *J. Yan[1], E. Bouchard[1], BZ. Chen[2].* 1) Department of Pediatrics, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, PQ, Canada; 2) Services of medical genetics, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, PQ, Canada.

The presences of interstitial telomere sequences (ITSs) located in the intrachromosomal sites were reported in many eukaryotic species as well as in humans. The occurrence of ITSs was proposed to be a consequence of telomere-telomere fusion during the evolution of human chromosomes or to be involved in chromosome rearrangements or chromosome instabilities. By means of double-strand PRINS, we discovered a specific ITS on human chromosome 22 at q11.2, where the region was found to contain rearrangement breakpoints for multiple disorders. This ITS appeared only in the PRINS technique but could not be detected by FISH using PNA probe. Moreover, it is present only in the product of one DNA strand extension primed by a synthesized oligonucleotide (TTAGGG)7 although another complimentary primer (CCCTAA)7 was used for the same samples in the double-strand PRINS technique. As the maximum frequency of ITS from parallel analyzed chromosome 21 was 10%, which means that no second positive ITS can be found in analyzed 10 metaphases, we defined a cut-off for negative ITS as 10%. Based on this definition, the positive cases that contained ITS at 22q11.2 were determined in 11 out of 16 (69%). The average frequency of the metaphases containing ITS at 22q11.2 in the 16 cases is 24.7%, which is significantly different from parallel scored chromosome 21 (2.9%). Research on the genome database found a DNA segment with high density of telomere-like tandem repeat sequence composed of 9-base monomers and located at 22q11.2. As this sequence spans 909 bp and is unique in scanned 12.5 Mb sequences covered from 22q11.1 to 22q12.1, we suggest that the observation of ITS signals at 22q11.2 by PRINS should correspond to the telomere-like sequence found in the genome database. Further study may provide important information of the ITS at 22q11.2 related to the genomic instability and the chromosome rearrangements.

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Molecular Ruler mapping and clinical correlations in seven patients with distal deletions of 9p. *G. Raca[1], X. Hauge[2], K. May[1], S. Cooper[1], R. Spiro[3], M. Adam[1], C.Lese Martin[1].* 1) Dept of Human Genetics, Emory Univ, Atlanta, GA; 2) Kennesaw State Univ, Atlanta, GA; 3) National Birth Defects Center, Waltham, MA.

Cytogenetically visible deletions of distal 9p have been reported in patients with cognitive impairment, trigonocephaly, characteristic facial features, hypotonia, cardiac abnormalities and abnormal genitalia. Previous studies have identified a critical region for the consensus phenotype to a region between 8 and 16 Mb from the 9p telomere, corresponding to bands 9p22.3 to 9p23. However, correlating genotype and phenotype findings has been difficult. Here, we report 7 new patients with deletions of the terminal portion of 9p. In one individual, the deletion was only detected by telomere FISH, while the six remaining individuals had cytogenetically visible deletions involving breakpoints in 9p23 or 9p24. Five patients had terminal deletions and two had unbalanced rearrangements involving additional material from another chromosome. Molecular Ruler clones, spaced 1 Mb apart and covering the most distal 14 Mb of 9p, were used as FISH probes to delineate the size of the deletions. In all 7 individuals, the deletion size was less than 14 Mb from the telomere, which is smaller than the majority of 9p deletion cases published in the literature. The smallest deletion was approximately 1 Mb and included at least 8 known genes. In order to develop genotype/phenotype correlations, the 6 individuals for whom clinical information was available were grouped into two categories based on their deletion size: 3 individuals had a deletion less than 7 Mb and 3 individuals had a deletion that was 10-14 Mb in size. Clinical evaluation and comparison showed little difference between these two groups for the physical features that were evaluated, suggesting that many of the genes contributing to the physical phenotype are located in the most distal 7 Mb of 9p. Gross motor skills appeared to be less severely impaired in the patient with the smallest deletion as compared to the remaining individuals. Language ability was uniformly poor, with sign language being the most common mode of communication in both groups.

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A variant Kabuki phenotype and malabsorption syndrome in a subject with *de novo* cryptic microdeletion involving 8q21.2 detected using whole genomic array CGH. *G. Novelli[1], M. Frontali[2], A.M. Nardone[1], A. Novelli[3], F. Gullotta[1], G. Palmieri[1], L. Biancone[4], F. De Nigris[4], A. Antoccia[5], A. di Masi[5], A. Sgura[5], E. Giardina[1], M. Biancolella[1], F. Amati[1], C. Tanzarella[5], F. Pallone[4].* 1) Biopathology Department,University of Tor Vergata,Rome,Italy; 2) Neurobiology and Molecular Medicine Institute,CNR,Rome,Italy; 3) CSS-Mendel Institute,Rome,Italy; 4) Department of Internal Medicine,University of Tor Vergata,Rome,Italy; 5) Biology Department,University of Roma Tre,Rome,Italy.

Kabuki syndrome is characterized by typical facial features,skeletal anomalies,dermatoglyphic abnormalities,short stature,and mental retardation,which may be accompanied by other congenital anomalies and health disorders.We describe a 22 yrs old male with a mild mental retardation,with malabsorption syndrome since he was 19 and showed several dysmorphic features.The main findings were short stature,large palpebral fissures with long eyelashes and arched eyebrows,large ears,micrognathia,hypodontia and excess of ulnar loops.Neurological examination showed nystagmus on lateral gaze.MRI scan revealed a marked dilatation of aqueduct and IV ventricle.A pattern resembling acute graft-versus-host disease,with apoptotic bodies and lymphocytes in crypts,was seen in the colon,with gland loss and interstitial fibrosis.Lab test showed a deficit of IgA and IgE.A provisional diagnosis of Kabuki make-up syndrome was made even if a few frequent features were lacking like inferior eyelid eversion.Using a 1-Mb genomic microarray,we found a cryptic microdeletion in the 8q21.2 region confirmed using microsatellite analysis and fluorescence *in situ* hybridization.The 8q deletion encompasses approximately 2 Mb of genomic DNA where the family of exonuclease GOR genes maps.We investigated the functional effects of GOR genes hemizygosity in patients fibroblasts examining cell cycle checkpoints perturbation and genetic instability after treatment with different DNA damage-induced agents.To study the telomere structure,telomere length was analysed with Q-FISH hybridization.We conclude that haploinsufficiency of GOR genes may play a critical role in determining some of the phenotypic features of the patient.

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MLPA: A Rapid and Sensitive Method for Detecting Copy Number Changes in 22q11.2. *G.R. Jalali, J.A.S. Vorstman, A. Hacker, B.S. Emanuel.* Dept. of Pediatrics, Div. Hum. Genet., Children's Hosp. of Phila., PA.

The 22q11.2 deletion causes a variable phenotype characterized by congenital and developmental abnormalities. About 87 % of deletions occur between Low Copy Repeats (LCR) A and D and 10% between LCR A and B or C. Sporadic deletions with atypical endpoints and other changes of 22q11 such as internal duplications, trisomy and tetrasomy have been observed. The diagnostic procedure often used for detection of copy number changes at 22q11.2 is Fluorescence In Situ Hybridization (FISH) with probes adjacent to the LCRs. The method is laborious and not sensitive enough to detect small deletions (40kb) within 22q11.2. A rapid, sensitive and less expensive method is necessary to detect these frequent 22q rearrangements. We have tested a commercially available MLPA kit (Multiplex Ligation-Dependent Probe Amplification) to assess copy number changes of 22q11.2. It is a novel method that allows the relative quantification of ~ 45 different target DNA sequences in a single reaction. The P023 MLPA kit has 9 probes specific for the 22q11.2 region. Multiple patients with variable deletion endpoints and other rearrangements of 22q11.2 have been characterized by FISH with cosmid or BAC clones. From this cohort numerous samples (50) were used in the current blinded study, including typical 22q11.2 deletions, various atypical deletions, duplications (trisomy and tetrasomy) and unbalanced translocations. MLPA findings were consistent with cytogenetic and FISH studies. No rearrangement went undetected. Repeated tests of the same samples gave consistent results. The number of false positive/negative calls correlated with the amount of variation within each sample (expressed as Standard Deviation, SD). At a SD in the range of 0.10 - 0.15, specificity and sensitivity were 0.96 (95%C.I.: 0.94 - 0.97) and 0.96 (95%C.I.: 0.90 - 0.98). At a SD below 0.10 both values improved (specificity: 0.99 (95%C.I.: 0.98 - 0.99) and sensitivity: 0.97 (95%C.I.: 0.94 - 0.98)). Thus, MLPA has proved to be a highly sensitive and accurate tool for detecting copy number changes in the 22q11.2 region, making it a fast and economic alternative to currently used methods.

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Development of a set of unique pericentromeric clones for the investigation of chromosome imbalance by array CGH. *S.A. Hornor[1], B.C. Ballif[2], B.A. Bejjani[1,2], L.G. Shaffer[1,2].* 1) Health Research and Education Center, Washington State University, Spokane; 2) Signature Genomics Laboratories, LLC, Spokane, WA.

Most regions of the chromosomes are subject to rearrangement, but certain genomic segments appear more susceptible than others. For example, the use of subtelomere probes has led to the identification of deletions and rearrangements in patients with unexplained mental retardation. In addition, pericentromeric regions of chromosomes seem to be more susceptible to deletion than other internal segments of the chromosome arms based on the identification of deletions in Williams, Potocki-Shaffer, Prader-Willi/Angelman, and DiGeorge/VCF syndromes with deletions in proximal 7q, 11p, 15q, and 22q, respectively. Based on the susceptibility of these regions to rearrangement, it is likely that deletions and duplications of other pericentromeric regions occur. However, little effort has been put forth to develop FISH probes to all unique pericentromeric regions. To provide these critical reagents, we constructed a set of clones to the 43 unique human pericentromeric regions (excluding the acrocentric short arms). Overlapping BACs were selected to cover ~5 Mb regions immediately distal to the most centric ~1.5 Mb of DNA, adjacent to the centromeres. Each clone was screened for sequences shared with other chromosomes based on set criteria. Selected BAC clones were evaluated by FISH mapping under constant conditions. Possible FISH outcomes included cross-hybridization, mis-mapped clones, and usable, locus-specific clones. The average number of clones found usable for each pericentromeric region was approximately 70%. These reagents promise to be of great value for clinical applications with existing and newly developing platforms. Array CGH provides a platform for efficiently screening the pericentromeric regions of all chromosomes to uncover novel imbalances and centric marker chromosome identification. The identification of deletions and duplications in the pericentromeric regions may delineate new syndromes or uncover the etiology of known clinical syndromes. In addition, chromosomal origins of markers will be identified and the extent of euchromatin delineated.

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Determination of a deleted region of an X-linked adreanal hypoplasia congenita with glycerol kinase deficiency.

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X-linked adrenal hypoplasia congenita [X-linked AHC, MIM#300200] is caused by mutations or deletion of the *DAX1* (dosage-sensitive sex reversal, adrenal hypoplasia congenita, critical region on the X chromosome, gene-1) gene. The *GK* (glycerol kinase) gene, responsible for hyperglycerolemia [glycerol kinase deficiency (GKD), MIM#307030], and the *DMD* (dystrophin) gene, for Duchenne muscular dystrophy [DMD, #310200]/ Becker muscular dystrophy [BMD, #300376], are mapped at centromeric region to the *DAX1* gene in Xp21 (Xpter-*DAX1*-*GK*-*DMD*-Xcen). About 1/3 of all AHC patients have the deletion of *DAX1*, *GK* and *DMD* genes as part of an Xp21 contiguous gene syndrome. Many of the patients are the Xp21 contiguous gene syndrome involving *DAX1*, *GK* and *DMD*.

We encountered a patient with X-linked AHC and GKD, but without clinical features of DMD. We performed fluorescent *in situ* hybridization (FISH) analyses using the BAC clones spanning the genes the *DAX1*, *GK* and *DMD*, and confirmed that this patient had a deletion including *DAX1* and *GK*, but had no deletion of the *DMD* gene. Some similar patients with AHC and GKD but without DMD had been reported. But, there are few reports described the precise breakpoints of the deleted region in such patients. We present the precise deleted region of this patient by molecular level.

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Identification of 5q35 microdeletion in a Korean patient with Sotos syndrome. *S.J Park, J.N Yoon, S.Y Jung, H.J Kim.* Dept Medical Genetics, Ajou Univ Sch Medicine, Suwon, Korea.

Sotos syndrome (OMIM #117550) is an autosomal dominant overgrowth disorder with developmental delay, typical dysmorphic craniofacial features, and advanced bone age. The syndrome is caused by haploinsufficiency of NSD1(nuclear receptor binding SET domain protein 1) due to microdeletion and point mutations. NSD1 consists of 23 exons with gene size of 160 kb and encodes a protein of 2,696 amino acids. The function is known to be involved in histone modification and regulation and maintenance of chromatin states. We identified 5q35 microdeletion including NSD1 region in one Korean patient with Sotos synrome. Microdeletion of the NSD1 were confirmed by FISH analysis. FISH was performed with two colour probes, the specific 5q35 BAC probe labelled with Cy3 and the 5q31 control probe with FITC. The specific 5q35 BAC probe was constructed using BAC clone DNA (NCBI build35). FISH was performed on metaphase chromosomes and interphase nuclei of cultured peripheral blood lymphocytes from Sotos patient and normal control individuals. In the Japanese patients with Sotos syndrome, most common mutations (50%) are microdeletion of 5q35 region including NSD1, while in the UK, approximately 70% of Sotos patients are shown to have NSD1 point mutations and only 10% of the patients have a microdeletion of 5q35 region. Therefore, further analysis will be studied to elucidate the mutation type of the NSD1 in Korean patients with Sotos syndrome.

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Severe non syndromic encephalopathy and microcephaly associated with Xq28 duplication in a male patient: MECP2 gene overexpression ? *C. Sibille, M. Ravoet, I. Abinet, Ch. Verellen-Dumoulin, N. Lannoy.* Centre de Genetique Medicale, Univ Catholique de Louvain, Bruxelles, Belgium.

Point mutations and large deletions in X-linked methyl-CpG-binding protein 2 gene(MECP2) have been found to be a cause of RETT syndrome in young girls. Mutations were also described in males patients having lethal encephalopathy with mental retardation. One large MECP2 genomic duplication was reported once in a female patient with preserved speech variant (PSV) suggesting a dosage effect of the protein. We present a 9 months old boy referred for mental and motor retardation, microcephaly (-2SD) and hypospadias. Weak dysmorphic features were present: bilateral anti-mongoloïd palpebral fissure with ptosis and wide ears. MRI showed a brain with reduced volume and dysmyelinisation. Echography of the heart showed small interatrial septum defect. At 5 years of age, his size was below the 3rd centile and head circumference below the 6SD. Clinical reports mentioned axial hypotonia. His mother is clinicaly normal but her half-brother died at 9 years old with major encephalopathy. Mother and son showed a normal karyotype without CGG expansion of FMR1 gene. However, multisubtelomeric FISH analysis identified in both a cryptic microdeletion of Xp22.3 (SHOX-, locus DXYS129-) associated to a duplication of Xq28 (locus ESTCdy16c07++, LAGE-1). Additional molecular analyses indicated that the Xpter deletion is confined within the PAR1 area while the duplication in Xq28 stretch over 3.200 kb (Xqter-DXS8061). Genomic duplication of the non-syndromic mental retardation MECP2 gene was shown by Multiplex Ligation-dependent Probe Amplification(MLPA). However, DNA of the mother using the (CAG)n polymorphism at the androgen receptor locus indicated that the abnormal X chromosome is preferentially inactivated. Moreover, quantitative RT-PCR showed a four fold overexpressed MECP2 mRNA in the peripheral blood mononuclear cells (PBMC) of the affected son versus normal control. We proposed that duplication of MECP2 gene might be implicated in a dosage-sensitive protein expression in major progressive encephalopathies accompagnied by mental retardation, microcephaly and severe encephalopathy .

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Detection of atypical deletions in the DiGeorge/Velocardiofacial region by array-based comparative genomic hybridization (Array CGH). *Z. Ou[1], J. Li[1], M.L. Cooper[1], H.M. Rosenblatt[2], M. Patel[1], S.W. Cheung[1], C. Chinault[1], A. Patel[1].* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.

DiGeorge /Velocardiofacial (DG/VCF) syndrome is the most common genomic disorder often associated a microdeletion on chromosome 22q11.2 region, resulting from non-allelic homologous recombination between sets of low copy repeats(LCRs). The main clinical findings include characteristic facial appearance, velopharyngeal insufficiency, learning disabilities, conotruncal heart defects and occasionally a cleft palate. A more severe subset of patients has a reduced or absent thymus gland and hypocalcemia. Of the 83% of patients with a detectable deletion, 90% had a common 3 Mb deletion in the 22q11.2 region while 7% had a smaller proximal breakpoint with a nested 1.5 Mb deletion. Rare patients with atypical deletions in the 22q11.2 region have also been observed. We have developed a BAC/PAC microarray for the detection of majority of the microdeletion syndromes including the DG/VCF syndrome. The clones in the DiGeorge/VCFS regions have been selected to identify 1.5 Mb, 3 Mb, and >4Mb deletions. Screening over 780 patients with our current V 4.0 clinical microarray, we identified 6 patients with 3 Mb deletion , 1 with a 1.5Mb deletion and one patient with 1 clone deleted just outside the common 3 Mb deletion locus. Patient with the 1.5 Mb deleted patient was a 35 week premature female infant, diagnosed with a membranous VSD and mild heart defect, hypocalcaemic seizures and absence of thymus function. Patient with 1 clone deleted distal to the common 3 Mb deletion was a twin (A) , born prematurely at 32 weeks gestation with truncus arteriosus, cryptorchidism and mild facial dysmorphism. Additional features included global developmental delay, failure to thrive with severe microcephaly. Twin B died of SIDS at 6 mos. of age and a half-sib died at 12 days of age with hypoplastic kidneys and polydactyly. Parental studies on both the atypical deletion patients are pending. Array-CGH provides quick and accurate detection of typical and atypical deletions/duplications in the DGS/VCFS region.

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Detection of genomic variations by array-CGH in 728 consecutive samples in a postnatal clinical cytogenetic diagnostics. *A. Patel, C. Shaw, P. Ward, K. Vonalt, M.L. Cooper, P.A. Lennon, P. Stankiewicz, C. Chinault, A. Beaudet, S.W. Cheung.* Dept Molecular & Human Genetics Baylor College of Medicine, Houston, TX.

Variations in the human genome of a few base pairs to a few hundred base pairs have been identified and correlations to health and disease have been studied. Genome wide identification of these variations has been hampered by a lack of high-resolution and high-throughput techniques. The development of array-CGH in recent years has provided such a tool for a rapid analysis of large scale human genome variation. Genomic variations of 250 Kb to over 1 Mb have been identified in normal individuals. Array-CGH is also quickly gaining acceptance in the clinical setting to identify disease causing genomic rearrangements and thus it becomes important to catalog and understand the clinical significance of these genomic variations. The Baylor College of Medicine Diagnostic Chromosome Microarray chip contains 366 FISH verified BAC/PAC clones spanning regions implicated in 55 known genomic syndromes and all the subtelomeric regions. In analysis of 728 patient samples by array-CGH for genomic rearrangements using a single reference male and female controls we found 47 patients with clinically significant and well characterized disorders. In addition, we also observed gains and losses of single clones in the 2qter, 4qter, 10qter, and 15q11.2 regions in 2-5% of the patients studied. Sequence analysis of these clones showed that they contained significant percentage of segmental duplications or had a high repeat sequence content. We also observed gain and loss of the region between BP1 and BP2 in the 15q11.2 region in 1.4% of the patients. Gain of genomic regions of the well-characterized microdeletion syndromes such as STS, SHOX, NF1 and NPHP1were also observed in less than 1% of the patients. For all of the above loci in at least 2 patients the gain or loss was confirmed in one of the parent. Array-CGH is proving to be a powerful tool in the analysis of genomic rearrangements but careful clinical correlations are needed to determine which genomic variations can be classified as polymorphisms and which may have a clinical significance.

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Array CGH identifies a partial trisomy 2p14p16 owing to an insertion in the chr 2 long arm. *J. Lapierre[1], B. Keren[1], M. Prieur[1], J. Amiel[1], D. Genevieve[1], M. Jacquemont[1], A. Tarrico[1], A. Munnich[1], S.P. Romana[1], A. Aurias[2], C. Turleau[1], M. Vekemans[1], D. Sanlaville[1].* 1) Genetics and Cytogenetics , Hopital Necker-Enfants Malades, Paris, France; 2) INSERM U509, Institut Curie, Paris, France.

Chromosome 2 short arm duplications are associated with a distinct phenotype. Here, we report a 5 years 6 months old girl presenting with intra uterine and post natal growth retardation, macrocephaly, facial dysmorphism, anteriorly displaced anus and mental retardation. Facial features include prominent forehead, narrow palpebral fissures, flat nasal bridge, short nose with anteverted nostrils, hypertelorism, bifid uvula, low set posteriorly rotated ears, large mouth with short philtrum and retrognathia. Hands were small and broad. Ophthalmologic was normal. No visceral malformations were found on cerebral MRI, abdominal and cardiac ultrasound. High resolution chromosome analysis revealed extra material at the 2q32 region. Fluorescent *in situ* hybridisation (FISH), using a whole chromosome 2 painting probe, showed that the extra material derived from chromosome 2, suggesting a 2q32q33 duplication. However, the clinical phenotype was discordant with previously reported cases with this imbalance. To further characterize this rearrangement, we used a 1 Mb CGH microarray. It showed that additional fragment correspond to the 2p14p16 region, from 53 Mb (RP11-469J7) to 64 Mb (RP11-494E18), with a suspected 600 kb deletion near the proximal breakpoint. Based on these results, we concluded that our patient had a 9Mb chr 2p insertion into the 2q32 region. Clinical presentation of our patient is more relevant to trisomy 2p than trisomy 2q in accordance with the array-CGH results. This observation underlines the accuracy of array-CGH to characterize complex chromosome rearrangement and to perform genotype phenotype correlations. Further studies are in progress to further confirm the small interstitial deletion. A mechanistic explanation of this complex rearrangement will be provided.

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Subtelomeric rearrangements in fetuses with congenital malformations: Two case reports. *J. Gignac[1,2,3], F. Tihy[1,2], E. Lemyre[1,2,3].* 1) Service de génétique médicale, Hôpital Sainte-Justine, Montréal, Québec, Canada; 2) Centre de recherche de l'Hôpital Ste-Justine, Montréal, Québec, Canada; 3) Faculté de médcine, Université de Montréal, Montréal, Québec, Canada.

Chromosomal anomalies are a frequent cause (20%) of fetal malformations. However, in over 50% of the cases, the etiology of these malformations remains unclear. This limits evaluation of the prognosis as well as the medical follow up of the affected fetuses. We are studying the implication of cryptic subtelomeric rearrangements in the development of fetal malformations. Subtelomeric analysis is performed by FISH on amniocytes cultured *in situ*. We present here two cases that we have identified through this study. In both cases, the fetus was diagnosed with severe malformations through ultrasound and had a normal karyotype. Subtelomeric analysis detected derivative chromosomes inherited from maternal translocations for both fetuses. In the first case, the foetus inherited a der(14)t(14;16). He presented gastro-intestinal, cardio-vascular, skeletal, lymphatic and cerebral anomalies as well as dysmorphic features. This phenotype correlates with the clinical descriptions of both 14qter monosomy and 16qter trisomy. In the second case, the fetus inherited a der(12)t(2;12). He presented renal, cardiac, cerebral and nucal fold anomalies as well as dysmorphic features. The clinical descriptions of 2qter trisomy reported in the literature do not include severe malformations other than cardiac anomlaies. Therefore, it is highly probable that 12qter monosomy is the cause of the abnormal phenotype of this fetus. To our knowledge, we provide here the first clinical description of 12qter monosomy. The detection of these subtelomeric rearrangements is important to understand the causes of congenital malformations and to better evaluate the prognosis of the affected fetuses. Moreover, this type of diagnosis has an extended impact on the families who will be offered appropriate genetic counselling. We now plan to characterize the breakpoints of these rearrangements with BACs in hope of establishing genotype-phenotype correlations. Support : *Fondation de l'Hôpital Ste-Justine.*

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Fluorescence in Situ Hybridization in Suspension Applied to Interphase Aneuploidy Quantification. *X. Wu[1], Z. Chen[2], J. Lucas[1].* 1) ChromoTrax, Inc., Arlington, VA; 2) University of Utah School of Medicine, Salt Lake City, UT.

Detection sensitivity of FISH performed on slides is 1 in 100-1000 cells. PCR is very sensitive, but lack of very accurate quantification still is a problem. By contrast, our innovative approach will greatly facilitate accurate and quantitative detection of chromosomal aberrations with potential detection sensitivity of 1 in 1,000,000 cells. Our technique includes a novel approach of hybridizing chromosomes in suspension with fluorescently-labeled DNA probes, in combination with flow cytometric method of analysis or magnetic sorting, in order to sensitively, precisely and rapidly quantify chromosomal aneuploidy, translocations and rearrangements. In our technique, DNA probes are hybridized to a suspension of cell nuclei or isolated whole chromosomes. The resulting hybridized cells or chromosomes are free-floating in solution, and may be subjected to flow analysis or magnetic sorting. In a pilot experiment, we demonstrated the efficacy of our approach by detecting aneuploidy in trisomy 21 human cells after solution hybridization with DNA probe specific for chromosome 21q. Our method produced staining the majority of nuclei with excellent detection of chromosomal domains, demonstrating its utility for clinical genetics. After hybridization in suspension of trisomy 21 cells with the above probe, essentially all free-floating interphase cells showed three distinct signals. In a direct comparison using trisomy 21 cells from four Down syndrome patients, FISH in suspension showed a slight increase in both sensitivity to detect three signals in interphase cells and hybridization efficiency compared to conventional FISH on slides. Scoring three signals in 500 cells per sample, we observed 1610 32 to 1506 29 for FISH in suspension to FISH on slides, respectively. Overall, FISH in suspension and on slides showed comparable hybridization results. Our revolutionary methods offer a new tool to assay chromosomes and DNA and provide the possibility to develop new techniques for analyzing low frequency of abnormal chromosomes based on FISH signals, potentially significant for early detection and diagnosis of genetic diseases and cancer.

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Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *E.A. Rorem, B.C. Ballif, C.D. Kashork, K. Sundin, V.M. Schmitz, B.A. Bejjani, L.G. Shaffer.* Signature Genomic Laboratories, LLC, Spokane, WA, USA.

One of the challenges of conventional cytogenetics is detecting low-level mosaicism for chromosomal abnormalities that may be responsible for the clinical phenotype. Recently, array CGH with its ability to screen the genome rapidly at an unprecedented resolution has revolutionized the cytogeneticists and clinicians approach to molecular cytogenetic testing. However, the limit with which array CGH can accurately detect low-level mosaicism is not known. Our laboratory uses the SignatureChip which we developed for the detection of microdeletions, microduplications, aneuploidy, unbalanced translocations, and subtelomeric and pericentromeric copy number alterations. This targeted BAC microarray contains 831 clones in 3-6 clone contigs covering 126 genetic loci and 104 control loci throughout the human genome. Of 1300 cases submitted to our laboratory for routine diagnostic screening by array CGH a substantial number of cases were identified as having mosaic chromosomal abnormalities. These include one case of a mosaic marker 18 (68% of cells), two cases of mosaic trisomy 8 (70% of cells and 28% of cells, respectively), one case of mosaic trisomy 9 (10% of cells), one case of mosaic trisomy 13 (21% of cells), and one case characterized by a mosaic deletion of the TWIST locus at 7p21.1 (6% of cells). Four of the six mosaic cases were known to have had previous or concurrent cytogenetic testing. Of these four, two had concurrent cytogenetic testing and were found to be abnormal after the microarray analysis had already been completed: the mosaic marker 18 (68% of cells) and one case of mosaic trisomy 8 (70% of cells). Two cases had previous cytogenetic analysis in other laboratories: one case of mosaic trisomy 8 (28% of cells) was detected cytogenetically by amniocentesis but was reported as pseudomosaic, and one case of mosaic trisomy 13 (21% of cells) was reported as normal. These cases illustrate the utility and comprehensive nature of array CGH in detecting a variety of chromosomal abnormalities and suggest that array CGH is a powerful platform for the detection of low-level mosaicism in clinical diagnostic specimens.

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Activation of DNA damage signaling by Shiga toxin in mammalian cells. *Y. Kabir[1], K.A. Talukder[2], S. Ahmed[2], A. Nur-E-Kamal[3].* 1) Kuwait University, Department of Family Sciences, College for Women, P.O.Box 5969,Safat-13060, Kuwait; 2) International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka, Bangladesh; 3) University of Medicine and Dentistry of New Jersy, Department of Pharmacology, Robert ood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA.

Ataxia telangiectasia (AT) is a rare autosomal recessive disorder due to defects in the ATM gene. Homozygous mutation in the ATM gene has been characterized by progressive cerebellar neurodegeneration, immunodeficiency, telagiectasia, radiation sensitivity and predisposition to cancer development. Mice heterozygous in the ATM gene have been reported to be susceptible to cancer. Recently, missense mutations have been found to be more prevalent in ATM heterozygous breast cancer patient. ATM is located primarily in the nucleus in dividing cells and involved in cell cycle regulation and maintenance of genomic stability. Shigella infection causes serious complications including CNS manifestation (seizures, and coma) and haemolytic uremic syndrome (HUS). It has been suggested that shiga toxin plays a critical role in developing clinical symptoms in Shegellosis. Shiga toxin (STX) has been shown to induce apoptosis in mammalian cells. Inhibition of protein synthesis by STX has been speculated to be the cause for induction of apoptosis. However, inhibition of protein synthesis by cycloheximide, which inhibits peptidyl transferase, is known to be anti-apoptotic. We have attempted to explore the molecular mechanism of shiga toxin-induced cell death. We have found that Shiga toxin activate DNA damage signaling (activation of ATM, H2AX and p53) in mammalian cells including a cerebellar granule neuron (primary) culture and induces cell death. STX is also known to deadenylate 28S ribosomal RNA (rRNA). We have also demonstrated that introduction of chemically synthesized RNA activates DNA damage signaling pathway and induces mammalian cell death. Results obtained in shiga toxin-induced activation of DNA damage signaling and apoptosis in mammalian cells will be presented.

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Microdeletion 13q12: a possible new syndrome identified using SNP screening. *P. Eydoux[1], S.L. Yong[2], N. Ali Akbarli[1], D. Bailey[3], A. Baross[4], M. Brown-John[4], M. Cao[3], S. Chan[4], D.L. Charest[4], A. Delaney[4], N. Farnoud[4], K. Garbutt[1], A. Go[4], G. Kennedy[3], M. Krzywinski[4], H.I. Li[4], S. Langlois[2], J. Schein[4], M.A. Marra[4], J.M. Friedman[2].* 1) Pathology and Medical Genetics; 2) Medical Genetics, Children's & Women's Hosp, Vancouver, BC, Canada; 3) Affymetrix, Santa Clara, CA, USA; 4) Genome Sciences Center, BC Cancer Agency, Vancouver, BC, Canada.

Array-based genomic screening is a recent and powerful strategy to identify allelic imbalances. Among these techniques, whole genome sampling assay (WGSA) using high-density SNP genotyping oligonucleotide arrays may allow identification of yet unknown microdeletions and microduplications. We report on a patient with developmental delay, growth retardation with delayed bone age, microcephaly, frontal bossing, synophris, short philtrum, retrognathia, dental crowding, small hands and hyperpigmentation of gums and nails. The patients karyotype was normal. Whole genome screening using the Affymetrix GeneChip Mapping 100K Assay was performed. Data analysis using the dChip software revealed a microdeletion within chromosome band 13q12, spanning approximately 5 Mb. We confirmed this deletion by FISH using BAC probes mapping to the potentially deleted region. To our knowledge, no such deletion has been reported so far. Arguments supporting a causal relationship between this chromosomal deletion and our patients disease include 1) the phenotype, compatible with chromosomal imbalance; 2) de novo occurrence of the deletion; 3) the size of the deletion; 4) evolutionary conservation of the deleted region; 4) the gene contents of the deleted segment. New cases are needed to further define the microdeletion 13q12 phenotype. SNP WGSA studies may serve as a tool for precise mapping of imbalanced regions and chromosomal breakpoints, allowing for accurate correlation between phenotype and genotype. Furthermore, with the help of FISH and other molecular techniques, SNP WGSA studies may help characterize new microrearrangement syndromes, individualize regions of uniparental disomy and identify genes involved in chromosomal rearrangements phenotypes.

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Two siblings with trisomy 9p derived from maternal translocation. *K.T. Abe, D.A. Cornélio, A.R. Benedetti, L.M. Formigli, N. Sakai Jr, A.F. Castro, I.M.P.O. Rizzo, C.E.S. Martins.* Laboratorio de Patologia-Citog, Rede SARAH de Hosp. de Reabilitação-Brasília-DF, Brazil.

INTRODUCTION: Characteristic clinical features of partial trisomy 9p include: mental retardation, short stature, hypertelorism, wide fontanelles, prominent ears, bulbous nose, down-turned corners of the mouth, hand and feet anomalies, simian crease, clinodactyly and hypoplastic phalanges of the fifth finger. More than 150 cases were reported with trisomy of many segments of the short arm of chromosome 9, and about 10% of these cases have entire or almost entire short arm involved in translocations. In the majority of these cases, the trisomic segment was transmitted from a parent carrying a reciprocal balanced translocation. Significant phenotypic variability occurs due to the various sizes of the trisomic segments and also due to concomitant involvement of other chromosomes. PATIENTS AND METHODS: Two siblings showing mental retardation, facial dysmorphisms, hand anomalies and congenital hip dislocation were examined. They were followed in our health service center since one was eleven day-old (patient 1) and the other one eleven month-old (patient 2). Nowadays, they are 8 year-old and 4 year-old, respectively. Chromosome analyses were performed by routine GTW-banding from peripheral blood on both patients and their mother (the father was dead). FISH study was performed using a whole chromosome painting (wcp) with a chromososme 9 and 22 specific probe and a chromosome 9 and 22 satellite probe. RESULTS: Chromosome analyses of the patients 1 and 2 showed 46,XY,+9,der(9;22)t(9;22)(9pter->9p10::22q10->22qter)mat,and 46,XX,+9,der(9;22)t(9;22)(9pter->9p10::22q10->22qter)mat, respectively,and the mother was 46,XX,t(9;22)(p10;q10). The GTW banding results were confirmed by FISH. DISCUSSION: The abnormal chromosomes in these cases were originated by adjacent I of 2:2 segregation during maternal gametogenesis. FISH performed on the metaphase of the patient 1 and his mother was helpful to characterize more precisely the familial translocation between 9 and 22. The clinical findings in our patients are similar to those reported cases described in the literature of trisomy 9p.

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Evaluating individuals with mental retardation using subtelomeric FISH and MLPA analyses. *Y.C. Li[1], C.C. Lin[2], M.F. Chao[2], S.J. Liao[1], L.J. Hsieh[2], M.M. Shiu[3].* 1) Dept of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Dept of Medical Research, China Medical University and Hospital, Taichung, Taiwan; 3) Institution of Biochemistry, Chung Shan Midical University. Taichung, Taiwan.

Unbalanced subtelomeric rearrangements have been identified in 2-10% of idiopathic mental retardation (MR). Approximately half of the subtelomeric MR cases are familial with some physical abnormalities. We have undertaken a FISH study using a panel of 43 chromosome arm specific subtelomeric probe mixtures (Vysis Inc.) and performed MLPA (SALSA P019 and P020 human telomere test kits; MRC-Holland Inc.) to screen a population of 44 MR patients selected from Nan Tou Retardation and Rehabilitation Center, Taiwan for subtelomeric aberration. The criteria for patient selection are severe to moderate MR, with family history of MR and normal karyotype by routine G-banding analysis. One patient out of the 44 patients studied (2.2%) was identified to have an unbalanced rearrangement involving subtelomeric region which was not readily identified by conventional G-banding. The aberration involved the deletion of a subtelomeric region in one of chromosome 19p which was replaced by a subtelomeric region from 16q. The patient has moderate MR and his mother also has mild MR with similar subtelomeric aberration. The subtelomeric deletion of 19p has not been reported and therefore the present case identified is novel. The study was supported by grants from the National Health Research Institute (NHRI-EX92-9207SI) and from the National Science Council (NSC93-2320-B-040-055), Taiwan.

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Persistent Trophoblastic Disease Following a Fetal Chimera Line (chi 46,XYpat[18]/47,XX,+mar1[8]) in One Twin. *M. Kolthoff, L. Hoffner, J. Dunn, T. Macpherson, J. Hunt, L. Sniezek, U. Surti.* University of Pittsburgh, Magee-Womens Hosp, Pittsburgh, PA.

A 25 year old female Gravida 2 Para 1 at 15.0 weeks with a dichorionic, diamniotic twin gestation presented to our Genetics Department due to abnormal obstetrical ultrasound findings. These findings included low amniotic fluid and poor growth for Twin A. In addition, Twin A's placenta appeared grossly enlarged and cystic. Twin B appeared normal as did its placenta. The patient developed heavy vaginal bleeding and cramping 48 hours later. She was diagnosed with an inevitable abortion and underwent a D&E procedure without complication. The pathological exam of Twin A's placenta showed large, hydropic villi with trophoblastic hyperplasia, consistent with a partial hydatidiform molar pregnancy. Both villi and skin cells from Twin A were analyzed and the karyotype showed two different cell lines: one biparental female cell line with an extra bisatellited marker chromosome derived from chromosome #13 determined by FISH and a second androgenetic 46,XY cell line based on DNA analysis. Paraffin FISH results also confirmed Twin A to be a fetal XX/XY chimera and Twin B results confirmed an XY conception. The maternal karyotype was found to be 48,XX,+mar x2.ish der(13)x2. Of note, the patient was of normal intelligence and without dysmorphic features. The patient's -hCG level remained elevated following the procedure. A pelvic sonogram showed a thickened endometrial lining with multiple cystic areas and vascular flow. Based on these findings, the patient was referred to gynecologic oncology for management of persistent trophoblastic disease. Methotrexate therapy was initiated and the -hCG levels have been undetectable since week 6. To the best of our knowledge, this is the first reported case of persistent trophoblastic disease following a fetal chimera. The most likely explanation for this phenomenon is the biparental and androgenetic genetic make-up of Twin A as based on our cytogenetic and DNA analysis.

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Severe dysmorphic features in a fetus with an unpredictable cytogenetic result. *A. Perez-Juana, M. Artigas-López, B. Hernandez-Charro, A. Alonso-Sanchez, M. Aristu, M.A. Ramos-Arroyo.* Genetics, Hospital Virgen del Camino, Pamplona, Navarra, Spain.

Chromosomal abnormalities are a common finding in early spontaneous abortions. About 25% of miscarriages are trisomic. Common trisomies (21,18,13) have a phenotype easily diagnosed by an experienced dysmorphologist. A healthy 32-year-old-pregnant woman went for her first obstetric sonogram at 12 weeks of gestation. A severe developmental defect of the lower pole of the conceptus was observed and sirenomelia was suspected. Fetal beat was absent and growth corresponded to a gestation of 9 weeks. It was the second pregnancy of a non consanguineous couple, they had a healthy son. Family history was unremarkable. The products of conception were submitted to our Genetics laboratory for evaluation. The fetus's length was 1,6cm and several malformations were observed. Its face was significant for a sloping forehead, marked hypertelorism, protruding eyes with absent lids, macrostomia but micrognathia, that reminded the canine facies of Neu-Laxova syndrome described in the Smiths Recognizable Patterns of Human Malformations. Limbs were rhyzomelic and remarkably short, polysyndactyly was present in left hand and both feet, an ectrodactyly on the right hand. An abdominal wall defect was present, anus was patent and no external genitalia were identified. The neck was short and an open neural tube defect covered approximately from the last thoracic to the first sacral vertebrae. After the dysmorphologic evaluation a tissue sample was obtained for further chromosome analysis. A thorough database research was realized while waiting for the cytogenetic result. Except for the EEC syndrome no other feasible diagnoses were found. Etiologically we considered two possibilities: a dominant syndrome resulting from a new mutation, subsequently with a negligible risk, or a rare recessive syndrome, giving a maximum recurrence risk of 25% for future pregnancies. The result of the karyotype came as a surprise for the dysmorphologists, revealing a full trisomy 18. In this case, the cytogenetic finding provided a valuable information and significantly reduced the prior risk of this couple.

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Cytogenetic and FISH studies on patients with recurrent spontaneous abortions. *C.W. Yu[1], C. Thompson[1], B. Cowan[2], W. Cleland[2], R. Hines[2].* 1) Dept Preventive Medicine; 2) Dept Obstetrics and Gynecology, Univ of Mississippi Medical Center, Jackson, MS.

Recurrent spontaneous abortions (RSA), defined as two or more consecutive pregnancy losses before 20 weeks of gestation, is one of the most significant issues in womens reproductive health, affecting an estimated 1-2% of all couples who wish to have children. The diagnostic evaluation of RSA is extensive, however in most cases, a clear cause is never found. Studies of one patient series concluded that the contributing factors for RSA could be grouped into five categories: anatomic, immunologic, hormonal, infectious and genetic. We report here the cytogenetic studies from 454 patients (both couples and individuals, ages ranging from 20 to 54) with a history of RSA losses from 1998 to 2004. Lymphocyte cultures were set up and harvested according to the routine laboratory procedures, and twenty G-banded metaphases were analyzed for each patient. If mosaicism of the X chromosome was suspected, an additional twenty to thirty cells were examined. Should the mosaicism still be indeterminate after the additional cells studied, a second blood specimen was requested for traditional cytogenetic as well as FISH studies. Eleven patients (2.4%) were found to have structural chromosome abnormalities; they are two Robertsonian translocations, seven balanced translocations, one inversion, and one mosaic marker chromosome. Forty-one patients (9.0%) had X chromosome mosaicism. Our data is compatible with the percentage of chromosome structural anomalies found in other reported studies. However, there are a greater percentage of patients who have X chromosome mosaicism in our study as compared to those in other reports. This may be attributed to the second blood study and the use of FISH for diagnosis. This study further demonstrates that the combination of tradition cytogenetics and FISH studies provide valuable diagnostic implements for patients with RSA.

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Whole genome array analysis of a patient with chromosome Y rearrangement, multiple congenital anomalies and speech and language delay. *C. Fawcett[1], A. Dawson[2], C. Harvard[1], Y. Qiao[1], E. Rajcan-Separovic[1].* 1) Dept. Pathology, Cytogenetics, UBC,Vancouver, Canada; 2) Cytogenetics, Health Sciences Center, Winnipeg, Canada.

The human Y chromosome is best known for its role in sex determination and spermatogenesis. Rearrangements of the Y chromosome have been frequently associated with infertility, and only sporadically is the rearranged Y noted in individuals with dysmorphic features and/or developmental delay (DD). In these instances, the possibility that Y chromosome rearrangements and the abnormal phenotype are coincidental can not be excluded. We have previously described a child with speech and language delay, short stature, mild dysmorphism and Duane abnormality who has two derivative Y chromosomes (Bal et al, 2004). Extensive molecular and cytogenetic studies showed a loss of the Yq chromosomal material distal to AZFa, the absence of Yp and Yq subtelomeric ends from both derivatives, and the presence of SRY on one of the Y derivatives. The proband has 2 male siblings who have speech and language delay but no other abnormalities and a normal chromosome Y. Because of the family history and the yet unknown role of chromosome Y deletions in DD/dysmorphic features, we have performed a whole genome array analysis using the 1Mb resolution array (Spectral genomics), looking for additional sub-microscopic rearrangements. Our analysis confirmed the loss of the Yq material in the proband, and the deletion of Yp region homologous to the Xp. These deletions were confirmed by FISH and are de novo in origin. In addition a loss of a single clone from 1q44, which was also deleted in the father but not in probands siblings, was noted. This clone has not been previously reported as a common polymorphism and may represent a rare deletion variant or possibly may be imprinted. Our report shows that the whole genome array analysis successfully elucidated the rearrangement of the Y chromosome and identified a rare deletion variant on 1q44. Cataloguing of rare variants and their molecular characterization in families with affected children may ultimately lead to better understanding of their role.

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Molecular cytogenetic characterization of an apparently balanced chromosomal rearrangement in an infant with severe craniosynostosis. *S. Shetty, K.M. Boycott, J.S. Parboosingh, F.P. Bernier, J.E. Chernos.* Dept Medical Genetics, Alberta Children's Hosp, Calgary, AB, and University of Calgary, AB, Canada.

Chromosome analysis by G-banded karyotyping is an important part of the investigation of children with physical and developmental disorders. When an unbalanced chromosomal rearrangement is identified, it is considered causative. However, when an apparently balanced rearrangement is detected, the causal link with abnormal phenotype is not so evident. We report an interesting case of craniosynostosis in a newborn girl with a clinically normal twin brother. All of her cranial sutures were fused and she had dysmorphic facial features. Routine cytogenetic analysis revealed a de novo balanced chromosome rearrangement: 46,XX,t(7;18)(p15.3;q11.2). Molecular cytogenetic characterization using Fluorescence In Situ Hybridization (FISH) with a panel of chromosome 7 BAC probes revealed a complex chromosome rearrangement with a refined breakpoint at 7p15.2 for the translocation and a cryptic interstitial deletion (7p21) distal to the translocation breakpoint. This deletion could be due to chromosome breakage secondary to the translocation or a concurrent breakage event. Candidate gene TWIST map to 7p21 region. Typically, craniosynostosis is associated with fusion of either coronal, metopic or saggital sutures. The greater severity of craniosynostosis in our patient may be attributable to a complete loss of candidate gene plus possibly other neighbouring genes leading to a unique contiguous gene syndrome. Microsatellite marker analysis will determine whether the locus encompassing TWIST gene is deleted and the role of TWIST gene will be looked into in our patient . Based on our experience with this case and recently published data a targeted molecular cytogenetics approach using BAC-FISH may be a useful adjunct to routine karyotyping for the detection of cryptic imbalances in individuals with an apparently balanced rearrangement and abnormal phenotype. The identification of an underlying genetic anomaly will improve diagnosis and genetic counseling for specific patients and contribute to a general understanding of the correlation between phenotype and genotype.

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Euchromatic duplication 8p23.1. A benign variant? *G. Sekhon, A. Lamb, J. Horwitz, K. Garcia, B. Ravnan, T. Morris, M. Klickovich.* Dept Cytogenetics, Genzyme, Santa Fe, NM.

We identified 11 families (6 pre- and 5 postnatal) of 8p23.1 duplications by cytogenetic analysis and further characterized by FISH with a chromosome 8 painting probe and locus-specific probes for D8S7, D8S574 and 8p subtelomere in the 8p23 region. Chromosome analysis follow-up of the prenatal families showed that, in one family, the duplication was of maternal origin, in two families was of paternal origin and was de novo in two families. In the remaining family the father was unavailable for the study, and thus the inheritance in the family remains inconclusive. Follow-up studies of parents in postnatal cases were performed in three of the families, one was maternal, one was paternal, and one was de novo. Parents were unavailable for follow-up in two of the postnatal families. FISH with a chromosome 8 painting probe (wcp8; Vysis) revealed that the extra material on the chromosome 8 was of chromosome 8 origin. FISH with the locus-specific probes showed two signals, indicating that 8p23.1 was duplicated on the aberrant chromosome. In the probands, where the duplication was inherited, one parent also showed an approximately double signal for the locus-specific probes and can be assumed normal variant. The literature suggests that 8p23.1 duplications are normal variants. However, recent findings also suggest that in some families cytogenetic duplications that appear similar to the euchromatic variants may be clinically significant. In the reported cases a parent carrying the duplication had phenotypic abnormalities. FISH showed that the interval from REPP-REPD was duplicated. If the duplication is inherited from a phenotypically normal parent, it can be assumed that it is a cytogenetic anomaly with no clinical significance. However, if inherited from a phenotypically abnormal parent or de novo, then FISH is warranted to rule out a duplication of REPP-REPD. Documentation with region-specific probes will clarify the clinical significance of this duplication. These studies will also help to reassure patients during prenatal counseling if a normal variant is demonstrated.

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A NEW CASE OF INTERSTITIAL 6q16.2 DELETION IN A PATIENT WITH PRADER-WILLI-LIKE PHENOTYPE AND INVESTIGATION OF SIM1 GENE DELETION IN 87 PATIENTS WITH SYNDROMIC OBESITY. *M.C. Varela[1], C.A. Kim[2], D.R. Bertola[2], A.Y. Simões-Sato[1], C.I.E.C. Fabris[1], C.P. Koiffmann[1].* 1) CEGH, Dept Genet and Evolut Biol, Inst Biosciences, Univ Sao Paulo, Sao Paulo, SP, Brazil; 2) Clinical Genetics Unit, Childs Institute, Hospital das Clinicas, Univ Sao Paulo School of Medicine, Sao Paulo, SP, Brazil e-mail: mcvarela@ib.usp.br.

The association of obesity, phenotypic abnormalities and mental retardation characterizes syndromic obesity. Its most common form is the Prader-Willi syndrome (PWS). A PWS-like phenotype (hypotonia, delayed psychomotor development, hyperphagia, obesity, mental retardation, small hands and feet) has been described in 4 patients with chromosome abnormalities involving the chromosome region 6q16.2 that includes the SIM1 gene. Herein we report cytogenetic and genetic studies, including a screening for the SIM1 gene deletion, performed on 87 patients with syndromic obesity and whose genetic tests were negative for PWS. All patients presented normal karyotypes, except for one who had a de novo deletion [46,XX,del(6)(q16.1;q21)]. Eighty-seven patients were analyzed with an intragenic polymorphic marker within the SIM1 gene: 74 cases presented biparental inheritance, 11 were uninformative and the deletion patient showed a paternal deletion. These patients with uninformative results were studied with other markers mapped to 6q16.2 region (D6S2418 and D6S475), and biparental inheritance was observed. In addition to the deletion of the SIM1 intragenic polymorphic marker, deletions of D6S2418 and D6S475 were also detected in our patient. This is the fifth case with an interstitial deletion of the chromosome segment 6q14-q21 presenting a PWS-like phenotype. In conclusion, deletions of chromosome region 6q14-q21 should be investigated in patients with hypotonia, developmental delay, obesity, hyperphagia, rounded face and full cheeks, strabismus, micro/retrognathia, small hands and feet and mental retardation, while SIM1 gene deletions or mutations should be investigated in individuals whose only characteristics are early-onset obesity and hyperphagia. Supported by: FAPESP, CEPID/FAPESP, CNPq.

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Dramatic instability of ring chromosome 5: Cytogenetic behavior. *D.J. Niccum, J. Lee, K.A. Casas, J.J. Mulvihill, S. Li.* Section of Genetics, Dept. of Pediatrics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK.

A ring chromosome is a rare genetic anomaly which may cause phenotypic abnormalities even without loss of genetic material. The ring chromosome induces difficulties in sister chromatid exchange by the breakage-fusion-bridge cycle at cell division, resulting in increased cell cycle arrest or apoptosis through the generation of secondary aneuploid cells. We present cytogenetically dynamic mosaicism of ring chromosome 5, including entangled, broken, doubled, or disrupted chromosome 5, as well as tetraploidy, in a 4 year-old male with developmental delay, growth failure, and dysmorphic features. The patient also had gastrointestinal problems, recurrent fever, and frequent upper respiratory infections. .

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Characterization of An Analphoid Supernumerary Marker Chromosome with A Neocentromere Derived from 15q2-->qter Using High Resolution CGH and Multiplex FISH Analyses. *X. Huang[1], M.I. Michelina[5], H.L. Mark[3], R. Harston[1], P.J. Benke[4], S. Price[2], A. Milunsky[1].* 1) Dept Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pathology, Boston University School of Medicine, Boston, MA; 3) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 4) University of Miami School of Medicine, Miami, FL USA; 5) Genetica S.C.R.L., Lima, Peru.

Supernumerary marker chromosomes(SMCs) without detectable alphoid DNA are predicted to have a neocentromere and have been referred to as mitotically stable neocentromere marker chromosomes(NMCs). We report the molecular cytogenetic characterization of a new case with analphoid NMC derived from15q25-->qter by using High Resolution CGH (HR-CGH) and multiplex FISH analyses with various alpha-satellite DNA probes, all- human-centromere probe(AHC),whole chromosome painting probes and a subtelomere probe. At age 3 months, the infant has been found to have bilateral sensorineural deafness. Chromosome studies showed that the propositus has a de novo extra SMC in 80% of cells examined. HR-CGH revealed rev ish enh(15) (q25qter). Molecular cytogenetic analysis and Molecular DNA polymorphism study demonstrated that this extra SMC is a NMC containing an inverted duplication of the distal long arm of chromosome 15(tetrasomy 15q25-->qter) which originated paternally i.e. ish der(15)(qter-->q25::q25[neocen]-->qter)(AHC-,CEP-,WCP15+, PCP15q x2)pat. This case represents a new report of a neocentromere on distal chromosome 15q suggesting that this region appears to be susceptible to the formation of neocentromeres.

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Prenatal diagnosis of 22q11.2 microduplication. *C. de La Rochebrochard[1,2], A. Goldenberg[2], I. Durand[3], A. Laquerriere[4], V. Ickowicz[5], P. Saugier-Veber[2], D. Eurin[5], H. Moirot[1,2], A. Diguet[5], F. de Kergal[4], C. Tiercin[1], S. Ferenbach[2], B. Mace[1], T. Frebourg[2], G. Joly-Helas[1,2].* 1) Laboratory of Cytogenetics; 2) Department of Genetics; 3) Department of Paediatric; 4) Department of Pathology; 5) Department of Obstetrics and Gynaecology, Rouen University Hospital, France.

The 22q11.2 microduplication syndrome is a rare recently recognized condition, which clinical phenotype includes subtle facial features, congenital heart defects and learning disabilities. We report here on a 23 year-old, gravida 3, para 1 woman referred at 23 weeks gestation. An obstetric ultrasonographic examination detected a severe, non conotruncal, complex congenital heart malformation. The parents elected to terminate the pregnancy at 24 weeks of gestation. RHG banded foetal chromosome analysis on the cord blood lymphocytes was normal. FISH, performed with probes for the DiGeorge syndrome (Vysis), showed three TUPLE1 signals in 70 out 103 interphase cells, with two chromosome 22 specific ARSA signals. Parental FISH analysis disclosed the same duplication on the healthy fathers interphase cells. Using a QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) assay specific of the 22q11.2 region, we estimated the duplication size to 3 Mb. Post-mortem examination showed an eutrophic female foetus presenting superior placement of eyebrows and downslanting palpebral fissures, long philtrum, micrognathism and dysplastic ears. Autopsy confirmed the cardiovascular findings (single atrium, total anomalous pulmonary venous return [TAPVR], small left ventricle, transposed great arteries and subpulmonary ventricular septal defect) and abdominal *situs inversus totalis*. It revealed thoracic heterotaxia. To our knowledge, this is the first case of a 22q11.2 microduplication prenatal diagnosis. Interestingly, TAPVR is a typical feature of Cat-eye syndrome associated with 22q11 tetrasomy. Phenotypic variability of the 22q11.2 duplication syndrome, ranging from none to severe congenital heart defects, and the technical difficulties in detecting microduplications strongly suggest that this syndrome remains under diagnosed.

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FISH analysis of sub-microscopic variability of the human genome. *Y. Qiao[1,2], C. Harvard[1,2], C. Fawcett[1], JJA. Holden[3,4], MES. Lewis[2], E. Rajcan-Separovic[1].* 1) Dept. Pathology, UBC, Vancouver, Canada; 2) Dept. Medical Genetics, UBC, Vancouver, Canada; 3) Dept. Physiology,Queen's University, Kingston, ON, Canada; 4) Dept. Psychiatry,Queen's University, Kingston, ON, Canada.

The variability of the human genome has been recognized since the introduction of chromosome banding analysis. It is now well-established that variation of chromosome band size in regions rich in repetitive sequences is not associated with a clinical phenotype. More recently, with the development and application of whole genome arrays, it is clear that the human genome also demonstrates tremendous variability at the sub-microscopic level, which is not restricted to regions containing highly repetitive DNA. We have used the commercial 1Mb resolution genomic array (Spectral Genomics) to identify novel sub-microscopic changes associated with autism, intellectual disability and cancer. During this process we identified recurrent changes in 60 affected and control individuals. In order to understand the sub-microscopic DNA copy number variability we are now using FISH to study copy number changes of polymorphic DNA clones and establish the pattern of their inheritance. Our analyses so far suggest the existence of several groups of polymorphic clones which map to interstitial regions: a) clones that show a wide copy number range (1~8 copies/chromosome); b) clones that show simple copy number variability (0-1 copies/chromosome); and c) clones which do not demonstrate changes by FISH, despite a gain/loss detected on the array, possibly due to the limited size of the sub-microscopic DNA change. The copy number changes for a group of selected clones were studied in 3 families and showed familial inheritance. Although at the present time microdeletions and microduplications found in normal individuals are thought to represent the natural variability of the human genome, the possibility exists that they may have future impact on an as yet unapparent, genetically influenced, disease susceptibility. Further studies of sub-microscopic polymorphisms, their cataloguing, inheritance and genetic significance promise to reveal exciting and new discoveries about our genome.

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Chromosomal Segregation in Spermatozoa of Fourteen Robertsonian Translocation Carriers. *E. Van Assche[1], G. Ogur[1], W. Vegetti[2], G. Verheyen[3], H. Tournaye[3], M. Bonduelle[1], I. Liebaers[1].* 1) Centre for Medical Genetics, University Hospital, Dutch-speaking Free University of Brussels, Laarbeeklaan 101, 1090 Brussels, Belgium; 2) First Department of Obstetrics and Gynaecology, University of Milan, Via della Commenda 12, 20122 Milan, Italy; 3) Centre for Reproductive Medicine, University Hospital, Dutch-speaking Free University of Brussels, Laarbeeklaan 101, 1090 Brussels, Belgium.

Robertsonian (Rob) translocations are one of the most common structural chromosomal aberrations observed in humans with an incidence of 1.23 per thousand births (Nielsen and Wohlert, 1991). In general, carriers of Rob translocations are phenotypically normal, but are at increased risk for spontaneous abortions and chromosomally unbalanced offspring. In addition, male carriers of Rob translocations can have fertility problems associated with abnormal sperm parameters. In the present study, the meiotic segregation behavior in spermatozoa from 14 Rob translocation carriers (7 der(13;14), 2 der(13;15), 2 der(14;15), 2 der(14;21) and 1 der(21;22)), with abnormal sperm parameters, were determined by Fluorescence In Situ hybridization (FISH). For the segregation analysis dual- and triple- color FISH, using sub-telomeric, and/or locus-specific and/or centromeric probes for chromosomes 13, 14, 15, 21 and 22 was used. Between 500 and 2 663 spermatozoa were analyzed per patient. Finally, spermatozoa of 5 carriers were also screened by FISH for enumeration of the chromosomes 13, 18, 21, X and Y for investigation of possible inter-chromosomal effects. It was shown that in each type of Rob translocation, meiotic segregation behavior is similar, comparable and occurs non-randomly. The majority of the spermatozoa result from alternate segregation (range: 76 % - 89.40 %). There is, however, still a considerable amount of unbalanced spermatozoa resulting from adjacent segregation mode (range: 10.30 %- 23.40 %). These data provide useful information for genetic counseling purposes. Moreover, aneuploidy for chromosomes 13,18, 21, X and Y was studied in five patients and suggested an inter-chromosomal effect.

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Single Chromosomal Copy Deletion Detection using the Affymetrix GeneChip Mapping Array in DiGeorge Syndrome. *C.M. Stanczak[1], Z. Chen[1], S. McGhee[2], S.F. Nelson[1], E.R.B. McCabe[1,2,3].* 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; 2) Department of Pediatrics, David Geffen School of Medicine at UCLA and Mattel Childrens Hospital at UCLA, Los Angeles, CA 90095; 3) Molecular Biology Institute, UCLA, Los Angeles, CA 90095.

DiGeorge syndrome is estimated to occur in 1:4000 births and is the most common contiguous-gene deletion syndrome in humans. Deletions in the 22q11.2 DiGeorge syndrome chromosome region (DGCR) prevent proper neural crest migration resulting in thymic and parathyroid hypoplasia and heart outflow tract defects. The purpose of this investigation was to determine whether the Affymetrix GeneChip Mapping 50K Array (SNP chip) could be utilized to detect and to map breakpoints in patients with DiGeorge syndrome. The SNP chip is a high-density oligonucleotide array that allows a rapid, standardized, parallel genotyping of over 50,000 SNPs across the human genome. Genomic DNA was isolated from three well-characterized Coriell cell lines and from a DiGeorge syndrome patients blood and analyzed on the Affymetrix platform. We confirmed the deletion breakpoints in the three cell lines and identified the patients deletion to be in the 22q11.2 DGCR. In all four cases we detected a single copy of genomic DNA in this region compared with two copies in normal individuals. The data permitted us to specify minimum and maximum deletion intervals for the patient and these intervals compared well with the control cell lines and corroborated the independent FISH results from this patient. This study demonstrates the use of the 50K SNP Chips for molecular cytogenetic analysis, and shows that they have utility beyond the genotyping which these arrays were initially designed. With approximately 1% of live births (40,000 U.S. neonates annually) having cytogenetic disorders, we envision a significant need for such a platform to facilitate rapid, high-throughput, genomic analysis for molecular cytogenetic applications.

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Megabase-scale variation at 8p23 inversion region indicative of genome hyperplasticity. *R.A. Ophoff[1,2], O. Lee[1], E. Slaten[1], J. Kung[1], N.B. Freimer[1,2], P.N. Rao[3].* 1) Ctr Neurobehavioral Genetics Univ California Los Angeles, Los Angeles, CA; 2) Department of Human Genetics, Univ California Los Angeles, Los Angeles, CA; 3) Molecular Cytogenetics Laboratories, Department of Pathology, Univ California Los Angeles, Los Angeles, CA.

The region of chromosome 8p23 was reported to contain a submicroscopical inversion polymorphism encompassing almost 4 Mb of unique sequence, flanked by large blocks of duplicated segments. This region has been implicated in susceptibility of severe bipolar as well as with panic disorder. In order to understand the possible role of the common inversion polymorphism in these neurobehavioral traits, our studies focused on the molecular characteristics and the genomic history of the 8p23 region. The segmental duplications flanking the inversion region are in opposite (palindromic) orientation mediating recurrent inversion events as seen by the multiple haplotypes associated with the inversion. Combined analysis by fluorescent in situ hybridization (FISH) and pulsed-field gel electrophoresis (PFGE) revealed extensive variation within these flanking duplicated sequences ranging from 100-1,500 kb in size. In a series of 30 African-American and Caucasian individuals from the Human Variation Collections of the NIGMS Repository, all subjects could uniquely be identified by PFGE showing distinctive alleles for each individual. Metaphase and interphase FISH analysis of the same individuals using the same sets of probes showed that both the proximal and distal segmental duplications are involved in these types of variations, and not necessarily linked to inversion status. Currently we are investigating two large CEPH reference pedigrees with known recombinants within the 8p23 region to test the meiotic stability of this type of genome plasticity, which may explain the extreme diversity seen in the African-American and Caucasian subjects. It is very likely that these megabase-scale variations in gene-rich regions have functional consequences that may be associated with human phenotypic variation.

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Delineation of deletion breakpoints in patients with monosomy 1p36 using a combination of Chromosomal Microarray Analysis and FISH. *S.-H.L. Kang, C.A. Bacino, J. Li, Z. Ou, C.A. Shaw, A. Patel, A.C. Chinault, S.W. Cheung.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Monosomy 1p36 is the most common human terminal deletion syndrome, with an estimated occurrence of 1 in 5000 live births. This chromosomal abnormality results in mental retardation, developmental delay, hearing loss, seizures, and cardiac anomalies, in addition to distinct facial features including large anterior fontanel, deep-set eyes, flat nasal bridge, asymmetric ears, and pointed chin. Previous analyses of 1p36 deletion syndrome patients revealed a large degree of variability in the location and size of the deletion. Furthermore, the molecular mechanism for generating these deletions still remains unclear. It is therefore important to finely map and characterize 1p36 deletion breakpoints in order to gain more insight into the molecular basis of the syndrome. We studied six patients, four with known 1p36 deletion syndrome based on initial karyotype and/or telomere fluorescence *in situ* hybridization (FISH) analyses, by an array-based comparative genomic hybridization technique known as Chromosome Microarray Analysis (CMA). Our version 4 clinical array contains eight non-overlapping BAC clones covering >10 MB of the distal end of chromosome 1p. The CMA analyses revealed three different classes of deletions. Three patients harbor telomeric deletions of sequences represented by four clones resulting in a loss of ~2-4 MB of genomic DNA; two patients harbor larger telomeric deletions of a region identified by seven clones resulting in a loss of ~7.6-8.7 MB of genomic DNA; and one patient harbors an interstitial deletion of a region detected by two clones resulting in a loss of ~0.5-3.0 MB of genomic DNA. Because the BAC clones on the microarray are not overlapping, we performed FISH analysis with overlapping BAC clones adjacent to the deleted regions detected by CMA in order to refine the deletion breakpoints. Further analyses of these genomic regions should aid in determining potential mechanisms for the generation of terminal deletions as well as improving genotype/phenotype correlations to locate candidate genes.

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Angelman syndrome in a boy with familial 15q11.2 microdeletion detected by microarray CGH. *O. Shimokawa[1, 2, 3], N. Miyake[1, 2], N. Harada[1, 2, 3], N. Sosonkina[4], T. Ohta[4], S. Saitoh[5], M. Iwakoshi[6], K. Yoshiura[1, 2], N. Niikawa[1, 2], N. Matsumoto[2, 7].* 1) Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 2) CREST, Japan Science and Technology Agency, Kawaguchi, Japan; 3) Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan; 4) The Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan; 5) Department of Pediatrics, Hokkaido University School of Medicine, Sapporo, Japan; 6) Nishinomiya Municipal Wakaba-en, Nishinomiya, Japan; 7) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

A familial 1.6-Mb-sized 15q11.2 microdeletion involving *UBE3A* was detected in a boy with features of Angelman syndrome (AS) by microarray CGH. Microarray using 2,173 BAC clone DNAs with 1.5-Mb resolution including subtelomeric regions, genes responsible for known mental retardation (MR)/multiple congenital anomaly syndromes was used for detection of genomic copy-number changes in children with idiopathic MR. The microarray detected a heterozygous deletion of RP11-1081A4 that covers *UBE3A* in a boy with features of AS, such as severe MR, absence of speech, epilepsy, happy disposition, abnormal EEG, sleep disturbance, microcephaly, prominent mandible, large mouth, and small, widely spaced teeth, but lacked characteristic laughter and hypopigmentation. Detailed FISH analysis revealed that his 15q11.2 deletion was 1.6-Mb in size, and involved *UBE3A, ATP10A* and a part of *GABRB3A*. FISH also revealed the same deletion in his phenotypically normal mother and maternal grandfather. Although FISH studies were unavailable in parents of the grandfather, it is likely that his deletion may have come from his father (great-grandfather).

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Single molecule, single cell detection *in situ*. *J. Koch, J. Lohmann, M. Stougaard.* Lab. of Molecular Pathology, Inst Pathology, Aarhus C, Denmark.

Established techniques of immunohistochemistry and *in situ* hybridisation enable the study of cells and biomolecules in their natural context. Thus, molecules are analysed inside the cells where they reside *in vivo*, and cells are analysed in their tissue context. Since molecules do not function by simply existing, but by interacting with and affecting other molecules, it is important to know where a molecule is, when it is there, and what other molecules are around. A simple example is an mRNA molecule with a mutation disabling its export into the cytoplasm. While such a molecule would exist, it would get not get beyond the nucleus, and it would not be able to mediate the synthesis of the appropriate protein. Likewise, any multi-cellular organism relies on communication between cells to control and modulate their function and behaviour. To understand what goes on inside a cell, it may therefore be necessary to know what other cells are present and what they do. A prime example is found in embryology, where a single cell develops into a great variety of vastly different cells depending upon what organ or tissue they end up in. Many pathological processes must be understood in this context as well, not least cancer, where the sick cells relies on ignoring the appropriate signalling and on producing their own signalling to fool the body into not eradicating them. Main limitations to *in situ* analysis relate to the sensitivity of the detection and the resolution of details. Thus, detection requires the summed up contributions of a number of molecules for them to be visualised, and small variations in the molecules, such as point mutations or splice variants in mRNA, generally escapes detection. We present new technology, capable of resolving minor molecular details in bio-molecules and of visualizing individual target molecules *in situ*. This is obtained with circular hybridisation probes and localised DNA synthesis in a rolling circle format, creating a tandem repeat covalently linked to the original hybridisation target. The circular probes can resolve point mutations in DNA and splice patterns in mRNA, and the rolling circle DNA synthesis produces enough copies of the probe to enable single probe molecules to show up in the microscope.

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Comparative human-mouse analysis of congenital diaphragmatic hernia (CDH) after genome-wide CGH using BAC microarrays. *D.A. Scott[1], M. Klaassens[5,6], S.A. Yatsenko[1], P. Stankiewicz[1], C.J. Fernandes[3], K.P. Lally[4], A. de Klein[5], D. Tibboel[6], W.W. Cai[1], M.J. Tsai[2], S.Y. Tsai[2], B. Lee[1,7].* 1) Dept Mol & Hum Genet, Baylor Col Med, Houston, TX; 2) Dept Mol & Cell Bio, Baylor Col Med, Houston, TX; 3) Dept Peds, Baylor Col Med, Houston, TX; 4) Dept Ped Surg, Univ of Texas Med School, Houston, TX; 5) Dept Clin Genet, Erasmus Med Centre, Rotterdam, The Netherlands; 6) Dept Paed Surg, Erasmus Med Centre, Rotterdam, The Netherlands; 7) Howard Hughes Medical Institute.

Congenital diaphragmatic hernia (CDH) is a common birth defect with variable morbidity and mortality. Most cases are sporadic making linkage based approaches to gene identification impractical. In an alternative approach, we used whole-genome microarray comparative genome hybridization (aCGH) to screen for CDH-related chromosomal regions that are duplicated or deleted in 15 infants with CDH and other non-pulmonary anomalies (CDH+). Infants with trisomy 13, 18 and 21 were excluded. Chromosomal anomalies were identified in five CDH+ patients (30%) and in two cases (13%) the chromosomal anomaly was identified by aCGH after a normal G-banded chromosome analysis. This demonstrates that chromosomal anomalies are a common cause of CDH+ and that inclusion of aCGH analysis can increase the detection sensitivity. Identified chromosomal abnormalities included Turner syndrome (45,X), mosaic trisomy 14q, duplication 6p21.3-p25.3, duplication 11q23-qter/deletion 12qter, and duplication 2q37-qter/deletion 15q26-qter. Deletions of chromosome 15q26 have been identified in individuals with CDH and a minimal deletion region for CDH that contains *COUP-TFII*, a transcription factor gene regulated by the retinoic signaling pathway, has been described previously. To determine if *COUP-TFII* plays a role in CDH, we generated mice that are homozygous for a targeted tissue-specific deletion of *COUP-TFII*. Mice that are homozygous for the *COUP-TFII* deletion have left-sided posterolateral CDH similar to the most common form of CDH in humans (Bochdalek-type). This strongly suggests that haploinsufficiency of COUP-TFII contributes to the development of CDH in individuals with 15q26 deletions.

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Common genomic polymorphisms in 24 psychiatrically normal controls. *S.L. Christian[1], H.L. Fritz[1], J. Conroy[2], N. Nowak[2], E.S. Gershon[1].* 1) Dept Psychiatry, Univ Chicago, Chicago, IL; 2) Roswell Park Cancer Institute, Buffalo, NY.

Whole genome screening using BACs in a microarray format, termed array comparative genomic hybridization (aCGH), has emerged as a valuable new tool to identify regions of gene dosage imbalance. This technique has the potential to identify many previously unrecognized deletions and duplications associated with human disease. However, to interpret these data common polymorphisms present in an unaffected population need to be identified. Several studies have examined normal populations, however, these subjects were not screened for psychiatric disorders. To apply aCGH to common psychiatric disorders such as autism, bipolar disorder and schizophrenia, a database of polymorphisms found in controls screened for psychiatric disorders is needed. In this study genomic DNA from 24 psychiatrically normal control subjects were analyzed using a 6k human BAC microarray to identify normal polymorphisms. An average of 4600 autosomal clones and 189 X chromosome clones were evaluated further for each subject. The average number of autosomal clones with abnormal dosage was 25.1 for < or > 3 SDs from baseline and 5.6 clones for < or > 4 SDs. Microsatellite confirmations showed mostly false positives using a 3 SD threshold. 91 different BACs showed abnormal dosage among these 24 subjects using a 4 SD threshold. 32 contain segmental duplications and/or map to other chromosomes using build 35. 3 clones contain gaps, 8 map to centromeres and 12 map to telomeres. A comparison with previously published reports showed 16 clones in common with Iafrate et al. 2004 and 9 in common with Sebat et al. 2004. 2 clones were in common between all 3 studies and 64 clones were unique to this study. Followup studies using a 19k human BAC microarray are in progress. Overall, the level of genomic polymorphisms identified in psychiatrically normal controls indicates the need for extensive evaluation of control subjects to interpret aCGH results on ill subjects.

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Maternal uniparental disomy 16 in a fetus with trisomy 16 mosaicism. *J. Puechberty[1], A.M. Chaze[1], V. Cacheux[1], P. Blanchet[1], C. Coubes[1], P. Lewin[2], G. Lefort[1], P. Sarda[1].* 1) Dept Medical Genetics, CHU Arnaud de Villeneuve, Montpellier, France; 2) Pasteur Cerba Laboratory, 95066 Cergy Pontoise cedex 9 France.

Second trimester serum screening in a gravida2 para1 34-year-old mother showed elevated beta-hCG levels indicating a Down syndrome risk of 1:10. Amniocentesis was performed at 18 WG. Karyotype analysis detected level III trisomy 16 mosaicism in a female fetus. A control amniotic fluid sample at 21 WG reported only 46,XX cells and concomitant scans were interpreted as normal. At 23 WG ultrasound noted short femora (<3rd centile) and the couple was referred for genetic counselling. The prospective parents were informed of the cytogenetic and ultrasound findings and offered fetal blood sampling and a third amniocentesis. Blood chromosomes were normal but amniotic fluid karyotype again showed trisomy 16 mosaicism At 28 WG scanning detected intrauterine growth retardation and a dilated digestive tract, suspected a heart defect and was unable to visualize the biliary duct. The couple elected to terminate pregnancy at 30 WG. Postmortem study confirmed most ultrasound findings and trisomy 16 mosaicism was found in placenta and various fetal tissues. Maternal [upd(16)] was diagnosed on the second amniotic fluid sample. The outcome of this pregnancy is discussed in the light of the double contribution of trisomy 16 mosaicism and maternal [upd(16)].

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EVALUATION OF CURCUMIN IN RADIATION INDUCED GENETIC DAMAGE IN MURINE CHROMOSOME STUDIES. *A. Corona-Rivera[1,2], L. Ruvalcaba-Ortega[1], C. Ortega-de la Torre[1], T. Montiel-Roldán[1], J. Vargas-Lares[1], R. Silva-Cruz[1], K. Contreras-Venegas[1], J.R. Corona-Rivera[1], M.A. Ramirez-Herrera[1], M.L. Mendoza Magaña[1], L. Bobadilla-Morales[1].* 1) Lab de Citogenética Genotoxici, Univ Guadalajara, CUCS, Guadalajara, Jalisco, Mexico; 2) Unidad de Citogenética, Hospital Civil de Belén Fray Antonio Alcalde, OPD.

Ionizing radiation induces chromosome aberrations and cell cycle alterations. On the other hand, it has been proposed that curcumin inhibits radiation-induced genetic damage detected with chromosome aberrations, micronuclei and comet assay. In this work we investigated the radioprotective action of curcumin trough observation of chromosome aberrations in mouse bone marrow cells.We worked with 34 Bal-C male mice of 6 weeks old divided in 6 groups. Mice were fed with curcumin for 5 days to different dosis and were irradiated with 6Gy at day 5. Femur bone marrow samples were obtained 24 hours after the irradiation. Group 1 was the negative control; group 2 was the irradiation positive control; group 3 was just fed a diet containing 0.1% of curcumin; group 4 received irradiation and were fed a diet containing 0.01% curcumin; group 5 received irradiation and were fed a diet containing 0.1% curcumin, and group 6 received irradiation and were fed a diet containing 0.5% curcumin. There were analyzed all groups versus control with chi-square test and there were found statistically significative decreases of damage in all groups except in fourth group. We propose that curcumin is able to protect against genomic damage induced by ionizing radiation in a dosage dependent manner.

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Uniparental disomies, micro-deletions and trisomies in spontaneous abortions. *S. Sonta[1], Q.Y. Li[1,2], R. Kimura[1], T. Ono[1], A. Makino[2], M. Tanemura[2], M. Sugiura-Ogasawara[2], K. Suzumori[2].* 1) Dept Genetics, Inst Developmental Research, Aichi Human Service Center, Kasugai, Japan; 2) Nagoya City University Medical School, Nagoya, Japan.

While the causes of about half of spontaneous abortions having a normal karyotype are mostly unknown, the fact that typical chromosomal abnormalities cause a large portion of spontaneous abortions suggests the possibility that functional, structural and constitutional abnormalities, such as uniparental disomy (UPD), micro-deletion, and abnormal X-inactivation, which are undetectable by usual chromosomal analysis, may serve to cause these abortions. To investigate such causes, polymorphic analyses of microsatellites were performed in 482 cases of spontaneous abortion with and without typical chromosomal abnormality. Of 482, 251 cases had various chromosomal abnormalities; they were trisomies, including 6 double trisomies and one triple trisomy, X-monosomies, polyploids, and others. The microsatellite analyses revealed that extra chromosomes of double and triple trisomies were all of maternal origin. Moreover, all extra chromosomes in each case occurred by nondisjunction at the same division, the first meiotic or mitotic division. These findings suggest the possibility that abnormal separation of two or more chromosomes may occur simultaneously in oogonia and ovum of aged women. In the remaining 231 cases with a normal karyotype, we found four cases with uniparental disomy: UPDs 14, 15, and 16, and partial UPD of chromosome 7. In analysis of microsatellite polymorphism on the X chromosome, on the other hand, polymorphic patterns of PCR products using some primer sets was not seen in two cases of 46,XY and one case of 45,X. We analyzed these 3 cases to confirm the deleted structure of the X chromosome in each case. The findings suggest the possibility that UPDs and micro-deletions may cause spontaneous abortions. This work was partially supported by Grant #16390481 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Identification of the DFNA44 hearing-loss gene. *M.A. Moreno-Pelayo[1], S. Modamio-Hoybjor[1], A. Mencia[1], R. Goodyear[2], I. del Castillo[1], G. Richardson[2], F. Moreno[1].* 1) Unidad Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain; 2) School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG.

Non-syndromic hereditary hearing loss (NSHHL), in which hearing loss is the only clinical symptom, has proven to be extremely heterogeneous genetically. Autosomal dominant forms account for up to 20% of cases with more than 40 loci (DFNA) now mapped and 20 genes currently identified. Recently, we mapped the DFNA44 locus in a Spanish family with postlingual progressive NSHHL. Here we have characterized the mutation responsible for DFNA44 and established the spatio-temporal expression pattern of the gene in the mouse inner ear. DFNA44 candidate genes were screened for mutations by heteroduplex analysis and direct sequencing of the coding region and exon-intron boundaries. The spatio-temporal expression pattern of DFNA44 protein in the mouse inner ear was investigated in cochlear sections from different developmental stages with affinity-purified rabbit antisera raised against two peptides at the C-and N-termini of the DFNA44 protein. The identified mutation consists of an 8-bp tandem duplication in exon 11 leading to a frameshift that replaces the last 15 amino acids of the human protein by a novel, 36 amino acid sequence. At embryonic day (E) 14.5, DFNA44 protein is expressed in the otic mesenchyme (MS), in the cells lining the lumen of the primitive cochlear duct, and in the nerve fibers of the spiral ganglion. At E17.5, DFNA44 is mainly localized in the MS. By postnatal day (P) 2, strong staining is seen in the apical region of the pillar cells (PCs), and a weak signal is seen in the outer hair cells and in the stria vascularis (SV). At P12 and P16, strong staining is seen in both PCs and weak staining is seen in the MS and SV. At P19, the staining is strong in the PCs, Deiter's cells and SV, but very weak in the MS. By P33 the protein is only detected in the SV. At all stages the protein is expressed in the epithelial cells of the vestibular maculae and in the three semicircular canals. The DFNA44 protein may play a crucial role in inner-ear development and auditory function given that its cochlear localization and expression levels vary dynamically with time.

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Large scale gene expression profiles of mouse early inner ear development. *S. Sajan[1], M. Warchol[2], M. Lovett[1].* 1) Departments of Genetics and; 2) Otolaryngology, Washington University, St. Louis MO.

Up to 30 million Americans suffer from some form of hearing loss, with one-third of those being between the ages of 21 and 65. While environment plays a role, genetic factors account for half of all hearing loss cases. Many of the genes that cause heritable deafness also play a role in the normal development of the inner ear. Only a handful of genes and pathways that affect this process are currently known, however. The mammalian inner ear consists of the hearing organ (the cochlea), and the balance (vestibular) organ. The latter contains three semi-circular canals for sensing angular acceleration, plus the utricle and saccule, both for sensing linear acceleration. To better understand the genetic program of these structures, we micro-dissected inner ear structures from mouse embryos at stages E9 to E15 at half-day intervals (covering the earliest discernible structures of the inner ear up until the development of true hair cells). Mouse Affymetrix gene chips were used to profile otic vesicles from E9 to E10, the rudimentary vestibular and cochlear organs from E10.5 to E12, and the saccule, utricle, and cochlea from E12.5 to E15. Two litters were obtained per time-point, each serving as a replicate. We used duplicate gene chips per time-point and identified ~1300 genes whose expression changed by at least 2-fold between any given stages of development. Among these were 15 genes known as important markers of inner ear hair and supporting cell development and differentiation. Some of these included *Atoh1*, *Hes5*, *Myo7a*, *Ush1c*, and *Gjb2*. Genes highly expressed in the cochlea, that had not been previously described, included *Forkhead box G1*, *Hey2*, *Irx3*, and *Hypothetical protein BC024118*. Among those highly expressed in the utricle were *Fgf7*, *Vanin1*, *Dlx1*, and *Rgs2*. In the saccule, four of the most highly expressed genes were *Gelsolin*, *Otoancorin*, *Claudin10*, and *Protocadherin8*. We validated many of these observations by RNA *in-situ* hybridizations. Finally, we employed bioinformatics tools to parse these genes into pathways and interactions based on function. One example of such a pathway (among at least 6 that we have identified to date) is the TGFB pathway.

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Characterization of a leucine-rich repeats containing protein selected from a human cartilage EST sequencing project. *S. Schlaubitz[1,2], C. Stelzer[2], B. Lee[1], B. Zabel[2].* 1) Dept. Hum. & Mol. Gen. & HHMI, Baylor College of Medicine, Houston, TX; 2) Childrens Hospital, Mainz/ Germany.

Starting from an EST sequencing project of 5000 clones from a human fetal cartilage library, we selected clones of interest that - after detailed bioinformatically analysis - seemed to have a putative function in cartilage and/or bone development and that were not been described yet. According to their putative protein domain structure, some of these selected clones are transcription factors that could have important regulatory functions in developmental pathways; while others show interesting expression patterns in cartilage and bone structures. One of these is a protein with four leucine rich repeats (LRRs) and a transmembrane domain (TMD) that shows similarities to several collagen binding proteins out of the family of small leucine-rich proteoglycans. Even though these proteins have additional C- and N-term. spec. recognition sequences and do not have a TMD that may function as an anchor for our gene of interest, the amino acid similarities within the LLRs are significant. In situ hybridization shows highest expression in proliferating chondrocytes of E13.5 mice embryos and a specific expression in the inner cortex of long bones of E17.5 mice. Northern Blot hybridization also shows an ubiquitously expression in newborn tissues, although the strength is not homogeneous. It is highly conserved both at nucleotide and amino acid level through a variety of organisms including human, mice, rat, C.elegans, drosophila and xenopus. Co-immunoprecipitation, performed by others, showed its interaction with vinculin, a gene product involved in the attachment of actin-based microfilaments to the plasma membrane. By fluorescence microscopy using antibodies and dyes for different compartiments and structures of the cell, we confirmed the co-localization with vinculin. We hypothesize that this gene might be involved in the structure of the microfilament network and could function as an anchoring plasma membrane protein able to interact with structural proteins. We expect that this function is specifically important in skeletal structures and are in progress to establish an animal model.

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Abnormal chondrocyte proliferation and differentiation in osteochondromas of Multiple Hereditary Exostoses patients. *C. Benoist-Lasselin[1], J.F. Mallet[2], A. Munnich[1], J. Bonaventure[1], L. Zylberberg[3], L. Legeai-Mallet[1].* 1) INSERM U393, Hopital Necker, Paris, France; 2) Service de Chirurgie Pédiatrique-CHU Caen-France; 3) CNRS FRE 2696, université Paris 7-Paris-France.

Multiple hereditary exostoses (MHE) is an autosomal dominant skeletal disorder caused by mutations in one of the EXT genes (EXT1 or EXT2), characterized by multiple osteochondromas that generally occur near the ends of growing long bones. Ext genes encode a type II transmembrane protein with glycosyltransferase activity. In MHE EXT gene haploinsufficiency is correlated with reduction of heparan sulfate in osteochondromas. In order to investigate the potential changes in chondrocyte proliferation or hypertrophic differentiation during endochondral ossification in sessile osteochondromas, exostoses samples from seven MHE patients were obtained for morphological and immunohistological studies. Indian hedgehog (IHH), Fibroblast Growth Factor Receptor 3 (FGFR3) and Parathyroid hormone receptor 1 (PTHR1) transcripts were detected in the pre/hypertrophic chondrocytes of osteochondromas and some Proliferating Cell Nuclear Antigen (PCNA)-positive chondrocytes were revealed with an anti-PCNA antibody. In addition, ectopic expression of collagen type I and other bone markers were observed in the cartilaginous osteochondromas, including osteocalcin (OC), osteopontin (OP) and bone sialoprotein (BSP). These data indicate that EXT mutations in cartilaginous osteochondromas induce an increased chondrocyte proliferation and a phenotypic change of chondrocytes. The differentiated cells fail to undergo apoptosis but instead exhibit bone-forming cell characteristics. We conclude that, defective heparan sulfate biosynthesis caused by EXT mutations in MHE maintains proliferative capacity and promotes transdifferentiation of chondrocytes into bone-forming cells.

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Necdin and MAGEL2: candidate genes for Prader-Willi syndrome. *R. Wevrick, J.R. Bush, M.A. O'Neill, A.A. Tennese, S.L. Kuny, C.L. Walker.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

The necdin and MAGEL2 genes are both inactivated in PWS, along with two other protein-coding genes (SNURF/SNRPN and MKRN3) and a gene for a set of regulatory RNAs (IC RNA and snoRNAs). Necdin and MAGEL2 are highly related proteins that share a conserved 189 amino acid MAGE homology domain. To determine the etiology of PWS, it is critically important to understand the role of necdin and MAGEL2 deficiency in humans, and to correlate findings in necdin-null and Magel2-null mice with the clinical and pathological findings in PWS. The necdin (Neurally differentiated Embryonic Carcinoma-cell derived factor) protein is up-regulated during terminal differentiation of neurons; necdin is also expressed in muscle and other tissues. Expression of Magel2 is developmentally regulated and becomes limited to distinct regions of the hypothalamus by the time of birth. Our new studies suggest that in response to specific developmentally regulated transcription factors, necdin is expressed and interacts with nuclear transcriptional repressor proteins that regulate the exit of neuronal precursor cells from the cell cycle. We also found that necdin and Magel2 act at the centrosome (microtubule organizing center) to assist conversion of this specialized cytoskeletal structure from one that forms a mitotic spindle in dividing neuronal precursors, to a highly organized structure that is essential for the development and elongation of bundled axonal tracts and for axonal transport in post-mitotic neurons. We propose that necdin and MAGEL2 are involved in the critical events whereby the transcriptional events that lead to cell cycle exit are coupled to the cytoskeletal events required for terminal differentiation. We have identified novel neurological developmental abnormalities of terminal differentiation and axonal outgrowth in necdin null mice, mirroring some of the neurodevelopmental abnormalities in PWS. In summary, our studies examine the contribution of loss of necdin and MAGEL2 function to the complex neurobehavioral outcomes in individuals with PWS. Research supported by CIHR, NSERC, and AHFMR.

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The Zic2 gene in pre and peri implantation mouse development. *S. Brown, L. Brown.* Dept OB/GYN, Columbia Univ, New York, NY.

Heterozygous mutations in Zic2 result in HPE and HPE-like malformation in mice, indicating that CNS development is quite sensitive to Zic2 gene dosage. Existing literature about the role of Zic2 in brain development suggests that it may act as a mediator of Sonic hedgehog (Shh) signaling in a manner analogous to Gli2 and other Gli genes. The fact that Zic genes and Gli genes are closely structurally related makes this plausible, but it is difficult to understand this model from the point of view of gene expression: Zic2 is expressed dorsally and does not overlap with Shh, which acts ventrally to pattern the forebrain and eye into paired structures. Another piece of evidence that argues that Zic2 acts earlier in development than Shh is the fact that a point mutation in Zic2 results in a more severe phenotype than that associated with loss of Shh. These observations taken together with our experimental observation that mis-expression of Zic2 during early development seems to lead to lethality at or before the time of implantation has led us to examine the expression of Zic2 in early embryos and in embryonic stem cells. We find that Zic2 is expressed in the inner-cell-mass (ICM) of E3.5- E4.5 blastocysts in a pattern similar to that of other genes such as Oct4, Sox2 and Nanog, known markers of pluripotentiality. Pluripotent ES cells, a tissue culture proxy for the ICM also robustly express Zic2. In-vitro differentiation into mature neurons results in down-regulation of Zic2. Although many Zic2 positive cells are present in cultures after neural differentiation, they do not express markers of neural, glial or astrocyte differentiation. However, Zic2 positive cells continue to express Sox2. We speculate that Zic2 may have a role in defining an early step in the path towards the development of a neural fate and that its role in the pre and preimplantation embryo may be relevant for later neural development.

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A functional genomic approach to find genes responsible for Holoprosencephaly. *M. Zollo[1], P. Carotenuto[1], L. Garzia[1], A.M. Bello[1], G. Vitale[1], N. Marino[1], C. Roma[1], N. Tata[1], A. Faedo[3], J. Rubenstein[3], S. Wilson[4], A. Bulfone[2].* 1) CEINGE, Biotecnologie Avanzate SCARL, Naples, Italy; 2) SCRI - HSR, Istituto Scientifico San Raffaele, Milan, Italy; 3) Nina Ireland Laboratory of Developmental Neurobiology, University of California at San Francisco, San Francisco, CA, United States; 4) Department of Anatomy and Developmental Biology, at University College London, UK.

Holoprosencephaly (HPE) is the most common congenital anomaly of the forebrain during embryogenesis. Our starting point strategy to identify new candidate genes for HPE is a collection of murine neuro-developmental highly expressed telencephalon genes. These transcripts which are unique and not available in public databases have been fully characterized (Bulfone and Zollo et al. 2005, in press). This approach allowed us to further select novel and rare transcripts involved in forebrain development, in the regulation of neural progenitor cell properties, including 48 new microRNAs. We will present data on 14 selected novel and rare transcripts including 2 novel microRNAs from this cDNA collection and candidates for HPE. To verify this, we are using the knowledge of brain development in Zebrafish animal model. A functional assay by interfering regular brain development using morpholino technology and/or overexpressing any of our candidates genes is now in progress and will give us clues on HPE potential involvements. Additionally an in vitro neurogenesis model such as P19, NSC and Neuro2A cells are carried out as mean of overexpression by creating stable clones, or down-regulation by using si-RNA technology. These experiments are performed in order to verify if any abnormal conditions will effect neural proliferation, differentiation, and specification, phenomena involved in HPE. Reference: TESS (Telencephalic Embryonic Subtractive Sequences): a Unique Collection of Neurodevelopmental Genes Bulfone and Zollo et al. Journal of Neuroscience 2005 (in press). Financing support: MIUR-FIRB RBA-U01RW82 grant (Ministero Italiano Università e Ricerca).

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Inhibitors of Differentiation (ID1, ID2, ID3 & ID4) genes are neuronal targets of MeCP2. *S. Peddada, J.M. LaSalle.* Med Micro & Imm, UC Davis Sch Med, Davis,CA.

Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder caused by mutations in MECP2, encoding methyl-CpG binding protein 2 (MeCP2). MeCP2, a predicted repressor of methylated genes, is hypothesized to be required for neuronal maturation by the regulation of multiple target genes. Identifying primary gene targets in either *Mecp2*-deficient mouse or human RTT brain has proven difficult. In order to identify primary targets of MeCP2 during neuronal maturational differentiation, a genome-wide expression microarray analysis was performed on human neuroblastoma SH-SY5Y cells induced to differentiate with and without transfection of a methylated wild type MeCP2 oligonucleotide decoy (MDWT) to disrupt the binding of MeCP2 to endogenous targets. The gene lists were analyzed using dChip for those transcripts with the highest fold changes and significant differences with differentiation that were specifically and significantly changed by MDWT transfection. Interestingly, of the genes showing significant expression differences with MDWT, all four members (ID1-4) of the same subfamily of helix-loop-helix proteins called ID genes (Inhibitors of DNA binding or Inhibitors of Differentiation) were identified as potential MeCP2 targets. *ID1*, *ID2*, and *ID3* expression decreased with differentiation while *ID4* increased, however, all four showed increased expression in MDWT transfected cells. Quantitative real-time PCR confirmed the increased expression of all four ID genes in MDWT transfected cells and *Mecp2*-deficient mouse brain compared to controls. Chromatin immunoprecipitation analysis confirmed MeCP2 binding to methylated CpG sites within *ID2* and *ID3* promoters in differentiated SH-SY5Y cells, suggesting that these genes are primary targets of MeCP2 during neuronal maturation. All four ID proteins were significantly increased in expression in *Mecp2*-deficient mouse and RTT human brain using immunofluorescence and laser scanning cytometric analysis. These results suggest that members of the ID family of cell differentiation regulators are ideal primary targets for MeCP2 regulation of neuronal maturation that can explain the molecular pathogenesis of RTT.

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Fate mapping *Pitx2*-lineage neurons in the developing heterozygous and homozygous mutant mouse brain demonstrates defects in neuronal location. *D.M. Martin[1,2], J.M. Skidmore[1], J.F. Martin[3].* 1) Pediatrics, University of Michigan Medical Center, Ann Arbor, MI; 2) Human Genetics, University of Michigan Medical Center, Ann Arbor, MI; 3) Alkek Institute of Biosciences and Technology, Texas A&M System Health Science Center, Houston, TX.

PITX2, a paired-like homeodomain transcription factor, is mutated in Rieger syndrome, a haploinsufficiency disorder affecting eyes, teeth, and umbilicus, with occasional cardiac and pituitary defects. Reports of individuals with Rieger syndrome and mental retardation or hydrocephalus have implicated *Pitx2* in central nervous system development. In mice, *Pitx2* is highly expressed in postmitotic neurons of the developing thalamus, midbrain, and hindbrain, and in the adult subthalamic nucleus, posterior hypothalamus, red nucleus, and deep layers of the superior colliculus. Our prior studies identified abnormal gene expression and neuronal projections in the subthalamic nucleus and superior colliculus in midgestation homozygous *Pitx2* null mice, but were limited by lack of reporter expression in wildtype or mutant cells. Here we report a *Cre/loxP* fate mapping approach for labeling *Pitx2* lineage neurons in heterozygous and homozygous mutant neurons in the central nervous system, using *Pitx2-Cre* knock-in mice and a previously characterized transgenic -actin conditional *lacZ* reporter strain. Mutant *Pitx2*-lineage neurons were severely reduced in the subthalamic nucleus, a basal ganglia region that is critical for suppressing unwanted movements and is overexcited in patients with Parkinsons disease. Mutant *Pitx2*-lineage neurons were ectopically placed in the *Pitx2* mutant embryonic superior colliculus, such that labeled cells were located deeper in the neuroepithelium in comparison to heterozygous null embryos. These observations suggest that *Pitx2* promotes regionally specific neuron formation and migration in the developing mouse brain, with potential secondary effects on axon outgrowth. Ongoing studies are directed toward identifying *Pitx2* cell fates in the embryonic and adult brain, and exploring the molecular mechanisms involved in *Pitx2* regulation of regionally specific neuronal differentiation and migration.

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Mice with a mutation in Rai1 display neurological phenotypes. *W. Bi[1], L.A. Yuva-Paylor[1], B.A. Antalffy[2], A. Goldman[3], J.W. Yoo[3], J.L. Noebels[3], D.L. Armstrong[2], R. Paylor[1], J.R. Lupski[1,4,5].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pathology, Baylor Col Medicine, Houston, TX; 3) Dept Neurology, Baylor Col Medicine, Houston, TX; 4) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 5) Texas Childrens Hospital, Houston, TX.

Smith-Magenis syndrome (SMS) is a mental retardation disorder associated with a ~ 3.7 Mb deletion in 17p11.2 and characterized by craniofacial features, developmental delay, and neurobehavioral abnormalities. Point mutations in retinoic acid induced 1 (*RAI1*), encoding a PHD zinc finger containing transcriptional regulator, were identified in nine published and one newly identified SMS patients without detectable deletion. All these patients displayed neurological anomalies including mental retardation, sleep disturbance, self-injurious behaviors, and two of them had seizures, suggesting that RAI1 plays a role in the nervous system. To further understand how mutations of *RAI1*, cause phenotypes in SMS, we generated mice with a null mutation of *Rai1*, and simultaneously inserted a *lacZ* reporter gene into the *Rai1* locus. Craniofacial abnormalities and obesity, which have been reported in SMS mouse models containing a hemizygous deletion, were observed in ~ 18% *Rai1+/-* mice. The vast majority of homozygous mice died during gastrulation and organogenesis. Heterozygous mice didnt show significant obvious behavioral, neurological abnormalities, or seizures, but they had abnormal electroencephalogram. The few surviving *Rai1-/-* mice displayed neurological phenotypes including hindlimb clasping, motor impairments, and electrographic seizures. Consistently, -galactosidase assays in the brain of *Rai1+/-* mice showed that the hippocampus and cerebellum were strongly stained and the staining was also observed in neurons in cerebral cortex, striatum, and thalamus. Our data further support that RAI1 functions in the nervous system, and *Rai1* mutant mice provide a useful tool for examining the expression pattern and for understanding the functions of this disease gene.

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Association of a putative mouse model candidate gene, GRHL3, with spina bifida meningomyelocele. *K.-S. Au[1], M.R. Dewhurst[1], T.J. Kirkpatrick[1], A.J. Jordan[1], J.M. Fletcher[1], I.T. Townsend[1], G. Villareal[3], G.H. Tyerman[4], S.M. Jane[5], S.B. Ting[5], T.M. King[2], H. Northrup[1,6].* 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Dept Int Med, Univ Texas Medical Sch, Houston, TX; 3) Dept Pediatrics, Baylor College of Med, Houston, TX; 4) Shriners Hospital for Children, Los Angeles, CA; 5) Bone Marrow Research Lab, Royal Melbourne Hosp, Melbourne, Australia; 6) Shriners Hospital for Children, Houston, TX.

Neural tube defects (NTDs) are a common birth defect with an overall incidence of 1-2/1000 live births in the United States. Spina bifida meningomyelocele (SBMM) (lack of closure of the spinal cord caudal to the head) represents a significant portion of NTDs. Of more than 100 known mouse NTD models, two [Axd (axial defects) and ct (curly-tail)] represent the best models for non-syndromic SB in humans. After communication with Dr. Jane in Melbourne, Australia, mutations of the coding regions in a putative candidate gene for the ct mouse were tested in 96 selected American Caucasian SBMM patients. Dr. Janes group created a grainy-head like 3 gene (Grhl3; also named Tfcp2l4) KO mouse that showed thoraco-lumbar-sacral SB. The Grhl3 gene is a mouse homolog of Drosophila grainy-head gene, a gene necessary for epidermal barrier wound repair in damaged Drosophila embryos. The Grhl3 gene is highly conserved and maps to the same region as the ct gene. The Grhl3 protein is a transcription regulator and functions as a master protein in many aspects of epidermal movement and fusion. We screened for mutations in the 18 known and 5 putative exons of the human Grhl3 homolog GHRL3 (also termed TFCP2L4) gene in DNA from 96 SBMM patients by sequencing. We identified 19 single nucleotide polymorphisms (SNPs) in the coding and non-coding regions flanking the exons. Nine of the 19 SNPs are not present in the dbSNP database. We are currently genotyping these SNPs in 100 Caucasian control samples and an additional SB study patients (~400) to evaluate whether any of these variants have alleles that may confer risk for development of SBMM. Significant allele frequency deviation from the control samples would suggest a possible role of the GRHL3 gene in SBMM formation.

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BRCA1 mutations show association with lesion location in spina bifida. *T.M. King[1], K.-S. Au[2], T.J. Kirkpactrick[2], J.M. Fletcher[2], K. Copeland[3], D. Francis[3], I. Townsend[2], G. Villareal[4], G.H. Tyerman[5], H. Northrup[2,6].* 1) Internal Medicine, University of Texas - Houston, Houston, TX; 2) Pediatrics, University of Texas - Houston, Houston, TX; 3) Psychology, University of Houston, Houston, TX; 4) Pediatrics, Baylor College of Medicine, Houston, TX; 5) Shriners Hospital for Children, Los Angeles, CA; 6) Shriners Hospital for Children, Houston, TX.

In the Brca1-deficient mice, 40% of the embryos had spina bifida and anencephaly with a disorganized neuroepithelium. We examine the relationship between two BRCA1 mutations to the position of the spinal lesion in patients with spina bifida meningomyelocele (SBMM). We examined the population structure of this gene in both Caucasians and Hispanics. Our affected case population consisted of 227 males and 182 females; 169 were Caucasian and 240 were Hispanic. Thirteen patients had lesions in the T10-T12 region, 98 patients had an L1-L4 lesion and 277 had a lesion at the L5 region and below. Twenty-one had lesions that overlapped more than one of the defined regions. We genotyped two intragenic microsatellite markers denoted BRCA1 F1 and BRCA F4 in our patients. Using ETDT, we observed over-transmission of the 164 allele in the BRCA1 F1 and the 133 allele in the BRCA1 F4 and under-transmission of the 170 allele in the BRCA1 F1 and the 139 allele in the BRCA1 F4. We used chi-square and ANOVA techniques. Lesion level was evaluated grossly and by specific vertebrae. Individuals with two copies of the 164 allele for BRCA1 F1 had SB lesions that were more caudal than individuals with the other genotypes. Individuals with one copy of the 139 allele of the BRCA1 F4 were likely to have lesions that were more rostral. From this, we conclude that the 164 BRCA1 F1 allele acts recessively and the 139 BRCA1 F4 allele acts dominantly. Compound carriers of BRCA1 F1 164/164 and BRCA1 F4 139/- had lesions that were equivalent to those individuals who carried the other genotypes, suggesting a counterbalancing effect. We conclude that BRCA1 participates in the phenotypic variability seen in SBMM.

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Ontogenetic changes in serum S100B in Down syndrome patients. *C.A. Goncalves[1], C.B.O. Netto[1,2], L. Portela[1], C. Kieling[3], U. Matte[2], T. Felix[2], T.R. Silveira[3], D.O. Souza[1], R. Giugliani[2].* 1) Biochemistry, UFRGS, Porto Alegre, RS, Brazil; 2) Genetics,UFRGS-HCPA, Porto Alegre,Brazil; 3) Paediatrics, UFRGS-HCPA, Porto Alegre, Brazil.

Introduction: Down syndrome (DS) is the most common genetic cause of mental retardation and most cases result from trissomy of human chromosome 21 and over expression of genes residing in the DS cricital region (DSCR). Some of the genes present at the DSCR express proteins commonly associated with neurodegenerative disorders, including APP, SOD-1 and S100B. Objectives: Investigate whether serum S100B protein concentration in DS children was increased and exhibited an ontogenetic variation, compared to control individuals. Material and Methods: Blood samples were collected from 48 DS patients (12-132 months) and 42 control subjects (6-120 months). All DS patients examined were confirmed to possess the chromosome abnormality (trisomy 21). Serum S100B protein levels were determined using commercial luminescence assay (BYK-Sangtec). Results: We observed significantly higher levels of S100B in the Down syndrome group (median of 1.354 mg/L) than in control group (median 0.38 mg/L). In agreement with the results obtained by Portela et al (2002), we also found a negative log correlation between serum S100B and age. However, there was no correlation ($p>0,05$) between the levels of serum S100B and age in the Down patients group. Discussion: This study indicates that S100B in Down syndrome patients is higher than in normal individuals, however this effect is not age- dependent, as it is in normal individuals. The more elevated and persistent extracellular levels of S100B in DS patients is present since the fetal period, possibly due to gene-dosage effect at beginning; this contributes to the general idea that S100B precedes amyloid lesions in Alzheimer disease. Support: FAPERGS, CNPq.

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Overexpression of SCN5A mutation N1325S is significantly linked to apoptosis of cardiac myocytes in the transgenic mice. *T. Zhang, Q.K. Wang.* Department of Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

Long QT syndrome (LQTS) is a cardiac disorder characterized by prolongation of the QT interval on electrocardiograms and ventricular tachyarrhythmias (VT), which may lead to syncope, cardiac arrest, or sudden death. Mutations in the cardiac sodium channel gene SCN5A are responsible for LQT3. We previously generated an animal model for LQT3 by transgenic overexpression of SCN5A mutation N1325S in the heart. The LQT3 mice manifested the clinical features of the LQTS including spontaneous VT and sudden death. However, the molecular mechanism for arrhythmogenesis needs further characterization. Increasing evidence supports the existence of apoptosis in the cardiac pathogenesis. Yet, its contribution to cardiac arrhythmogenesis is not clear. In this study, we examined the myocardium of LQT3 mice for the presence of apoptosis. Cardiac apoptosis was analyzed at a continuum of ages using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. Compared to wild type littermate control mice, the LQT3 mice displayed a significant increase of apoptotic cardiomyocytes ($p<0.001$). We further found that cardiac apoptosis in the LQT3 mice is age-dependent with older mice developing a significantly higher level of apoptosis. To the best of our knowledge, this is the first time that cardiac apoptosis has been detected in LQTS. Our results suggest that apoptosis may play an important role in the pathogenesis of LQTS and may be a novel mechanism for arrhythmogenesis.

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Knockdown of Sterol Co-A Destaturase (SCOD) causes craniofacial defects. *S. Beiraghi[1], J.J. Essner[2], J.D. Larson[1], S.A. Wadman[2], A. Nasevicius[2], M.C. Eide[2].* 1) Arnold & Mabel Beckman Center for Transposon Research, University of Minnesota, Minneapolis, MN; 2) Discovery Genomics, Minneapolis, MN.

Disruptions in the human *Acyl Co-A Destaturase 4 (ACOD4)* gene is associated with cleft lip and palate. There are two genes similar to human *ACOD4, zebrafish zACOD* and *zSCOD*, both expressed in the zebrafish embryo through 7 days-post-fertilization (dpf) as determined by RT-PCR. **Objective:**The objective of this study was to investigate the function of *ACOD4* during craniofacial development using zebrafish as a model system. **Method:** Morpholino phosphorodiamidate oligonucleotide (MPO)-based targeted gene knockdowns of *zACOD* and *zSCOD* were performed to determine the function of these genes during early zebrafish development. Defects in craniofacial development observed by alcian blue staining of the cartilage. Analysis of early neural crest migration and specification in the *zSCOD* knockdown embryos performed by *in situ* hybridization with *crestin, distalless2, forkhead6, and fgf3*. **Results:** While *zACOD* did not show a localized expression pattern by *in situ* hybridization, *zSCOD* was observed in the zebrafish central nervous system (CNS) from 1 dpf through 3 dpf. Knockdown of *zACOD* resulted in embryos that displayed normal development. In contrast, the knockdown of *zSCOD* caused severe defects in craniofacial development. The observed defects included reversal of the ceratohyoid cartilage, lower jaw anomalies, and branchial arch defects. Analysis of early neural crest migration and specification in the *zSCOD* knockdown embryos by *in situ* hybridization with *crestin, distalless2, forkhead6,* and *fgf3* revealed normal expression patterns, indicating that the craniofacial defects arise subsequent to these early steps. **Conclusion:** While the knockdown of zebrafish *zACOD* does not appear to be similar to patients with mutations in *ACOD4*, the knockdown of zebrafish *zSCOD* results in defects of the cranial skeleton and indicates a conserved role for this gene family in craniofacial patterning.

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Disruption of *NFIA* is associated with neural tube defects and CAKUT. *W. Lu[1,4], F. Quintero-Rivera[2], Y. Fan[1], H.L. Ferguson[1], F. Alkuraya[1], A.W. Higgins[1], R.E. Peters[1], R.M. Gronostajski[5], C.G. Campbell[6], B. Rilliet[7], P. Parvex[7], D.J. Harris[3], A.H. Ligon[1], B.J. Quade[1], G.A.P. Bruns[3], J.F. Gusella[2], C.C. Morton[1], R.L. Maas[1].* 1) Brigham & Women's Hospital, Boston, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Childrens Hospital, Boston, MA; 4) Boston University Medical Center, Boston, MA; 5) State University of New York at Buffalo, Buffalo, NY; 6) Children's Hospital of Western Ontario, London, Canada; 7) University Hospital, Geneva, Switzerland.

Congenital anomalies of the kidney and urinary tract (CAKUT) are a family of diseases with a diverse anatomical spectrum, including renal dysplasia and vesicoureteral reflux (VUR). About 1% of human fetuses have CAKUT, which are the primary cause of end stage renal failure in children. Neural tube defects (NTD) are commonly associated with nephro-urological complications such as neurogenic bladder and renal insufficiency. Despite their association, little is known about the developmental link between CAKUT and NTD and the molecular pathogenesis of these disorders. To identify genes that cause CAKUT, as part of the Developmental Genome Anatomy Project (DGAP), we studied a patient (DGAP104) with bilateral dysplastic kidney and VUR. This patient has a balanced chromosomal translocation, 46,XX,t(1;20)(p31.3;q13.31), and a NTD including congenital hydrocephalus, thin corpus callosum, Chiari I malformation, tethered spinal cord, and a low vertebral deformity. Using fluorescence *in situ* hybridization, we identified the 1p31.3 breakpoint within intron 2 of *NFIA*. *NFIA* belongs to the nuclear factor I (NFI) family of transcription factors that function both in regulation of adenoviral DNA replication and cellular gene expression throughout development. Tissue *in situ* hybridization revealed *Nfia* to be highly expressed in the developing mouse kidney, brain and ventral ependymal layer of the neural tube. *Nfia* knockout mice show hydrocephalus, agenesis of the corpus callosum, and dysplastic kidney with incomplete penetrance. Analysis of *NFIA* in familial NTD with renal defects is underway to test the hypothesis that dysregulation of *NFIA* is pathogenetic some cases of NTD and CAKUT.

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Neural crest deficit in craniofacial skeleton precursors of Ts65Dn mice, a model for Down syndrome. *R.J. Roper, R.H. Reeves.* Dept of Physiology, Johns Hopkins Univ Sch Med, Baltimore, MD.

Ts65Dn mice are trisomic for orthologs of about half of the genes found on human chromosome 21 and exhibit several phenotypic similarities to Down syndrome (DS). Dysmorphology of the craniofacial skeleton, a distinguishing feature found in all DS individuals, is also seen in Ts65Dn mice. Because many tissues affected in DS have a neural crest cell (NCC) component, it has been hypothesized that trisomy 21 causes a defect in NCC, but no direct experimental evidence supports or refutes this hypothesis. To test whether trisomy affects NCC and contributes to the etiology of DS craniofacial dysmorphology, we crossed Ts65Dn mice to mice homozygous for the *Wnt1-lacZ* transgene which is expressed in NCC at embryonic day 9.5 (E9.5). E9.5 Ts65Dn x *Wnt1-lacZ* offspring were age matched by somite number, and trisomic and euploid embryos identified by FISH analysis of the yolk sac. At E9.5, we saw no selection against or gross developmental delay of trisomic embryos. Unbiased stereology revealed a significantly reduced NCC number and volume of the 1st pharyngeal arch (PA1), the mandibular precursor, in Ts65Dn vs. euploid littermates. Fewer NCC were found between the neural tube and PA1 in Ts65Dn embryos, suggesting a deficit in NCC generation or migration. Additionally, a reduction in PA2 NCC in Ts65Dn embryos implied a generalized NCC defect with trisomy. This is the first direct demonstration that trisomy affects NCC and supports further investigations into the etiology of the NCC deficit and its contribution to mandibular hypoplasia in Ts65Dn mice and DS.

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A novel hypothesis for the development of gastroschisis in humans: embryologic basis and implications for research. *M. Feldkamp[1], J.C. Carey[1], T. Sadler[2].* 1) Pediatrics, University of Utah, Salt Lake City, UT; 2) Twin Bridges, Montana.

Objective: Gastroschisis is a ventral body wall defect whose embryological origin remains an enigma. Four mechanisms have been proposed to describe how gastroschisis occurs, with the most recent of these, the vascular disruption hypothesis, most widely accepted. We present a new hypothesis to explain the developmental basis of this defect. Background: Four folds are essential for ventral wall closure which draws down in a purse string effect around the umbilical region. The edge of this area is the future umbilical ring, the transition zone between embryonic ectoderm covering the body wall and the amnion. Passing through the ring are the connecting stalk containing allantois, umbilical vessels, and the canal connecting the intra- and extraembryonic cavities, and the yolk stalk (vitelline duct). At 27 days post-conception, the vitelline duct diameter is narrower than the connecting stalk, but the 2 structures remain as separate entities. Early in the 5th week, the gut tube begins to lengthen to form the primary intestinal loop that remains attached to the yolk sac via the vitelline duct. Also, in the 5th week, the vitelline duct and connecting stalk are in close proximity until they merge to form the umbilical cord. A vitelline duct remnant results in a Meckels diverticulum. A Meckels diverticulum occurring with gastroschisis would require the bowel to prolapse prior to the merging of the vitelline duct and connecting stalk. Conclusion: We posit that gastroschisis is a defect involving altered embryonic folding of the ventral body wall before the end of the 5th week. The presence of a Meckels diverticulum in cases supports this hypothesis. This hypothesis suggests an earlier origin than other and has implications for designing studies of etiology and candidate genes.

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Molecular and expression analysis extends the phenotypic spectrum of GLI3 mutations to agnathia and oligosyndactyly. *S. MOUGOU-ZRELLI[1], S. AUDOLLENT[1], J. MARTINOVIC[1], M. LE MERRER[1], A.L. DELEZOIDE[2], C. OZILOU[1], L. DEVISME O. BOUTE[3], D. SANLAVILLE[1], A. MUNNICH[1], F. RAZAVI[1], M. VEKEMANS[1], T. ATTIE-BITACH[1].* 1) Genetics and INSERMU-393, Hopital Necker, Paris; 2) Biologie du développement, Hopital R. Debré, Paris; 3) Génétique et foetopathologie, CHU de Lille, France.

GLI3 mutations lead to Greig cephalopolysyndactyly (GCPS), Pallister-Hall (PHS) and postaxial polydactyly type A/B. PHS first described as a neonatally lethal condition associates hypothalamic hamartoblastoma (HH), post axial polydactyly (PD) and imperforate anus (IA).GCPS is less severe and combines polydactyly and craniofacial features. Recently, a phenotype-genotype correlation has been shown both for the location and the nature of GLI3 mutations, suggesting different roles of GLI3 during development. Here we report on a molecular study of GLI3 in 15 children referred for GCPS or PHS, as well as in 20 fetuses selected for either HH and PD (8) or IA plus 2/5 features of the PHS spectrum (12) i.e. growth retardation (IUGR), micropenis, limb, heart, or renal anomaly. Most of the latest were VACTERL cases. We identified a heterozygous GLI3 truncating mutation in 6/15 children (5 GCPS and 1 PHS) and 3 fetuses, as well as a large GLI3 gene deletion (GCPS). All mutations were novel and consistent with the genotype-phenotype correlation. Among fetuses, one typical PHS was confirmed. In another, the association of IUGR, PD, bilateral renal agenesis without HH or IA led first to the suspicion of Smith-Lemli-Opitz (SLO). The third fetus had a severe and unusual phenotype with agnathia and absence of oral orifice, oligosyndactyly of 4 limbs, HH, IA and absence of kidneys, vagina, adrenals, pituitary gland and corpus callosum. Microarray CGH analysis (1 Mb) was normal. In situ hybridisation of GLI3 confirmed its early expression in human pharyngeal arches then mandible. No mutation was identified in cases with HH and PD as part of another syndrome (ODFVI, Meckel, short ribs-PD) or in VACTERL cases. Our results emphasise on the possible lethality of GLI3 mutations during development, extend the phenotypic spectrum to severe craniofacial and reductional limb defects and show the overlap between PHS and SLO.

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Severe growth retardation and perinatal death of mice with disrupted *Nedd4* gene. *P. Shi[1], X. Cao[1], N. Lill[2], P. Kirby[3], E. Sweezer[1], J. Van der Veer[1], T. Kinney[1], K. Volk[4], J.B. Stokes[4,5], B. Yang[1].* 1) Obstetrics & Gynecology, University of Iowa, Iowa City, IA; 2) Pharmacology, University of Iowa, Iowa City, IA; 3) Pathology, University of Iowa, Iowa City, IA; 4) Internal Medicine, University of Iowa, Iowa City, IA; 5) VA Medical Center, Iowa City, IA.

Nedd4 (neural precursor cell expressed, developmentally down-regulated 4) is ubiquitin ligase. There are two isoforms in mouse, the ubiquitously expressed Nedd4, and Nedd4-2, which has more restricted (liver and kidney) expression. Nedd4-2 interacts with and negatively regulates epithelial sodium channel. The Nedd4 function is unknown and it is thought to be involved in the down-regulation of growth factor receptors, including insulin-like growth factor-I receptor, through the interaction with Grb10 (growth hormone receptor binding protein). The aim of this study was to elucidate the function of this protein. Mice with *Nedd4* disrupted were generated from ES cells obtained in the gene-trap experiment. At birth the body weight of -/- mice was 1/3 of that of +/+ littermates and -/- mice died right after birth. Immature or delayed development was seen in lungs, skeletal muscle (including diaphragm), skin and spinal cord in -/- newborn. Mouse embryonic fibroblasts (MEFs) isolated from -/- embryos showed reduced mitogenic activity relative to +/+ MEFs, as reflected by their decreased rate of growth in 10% serum and reduced colony formation following low density seeding. The percentage of cells at G0-G1 phase was significantly increased, while the percentages of cells in G2-M and S phases were significantly decreased in -/- MEFs compared to +/+ MEFs. Although +/- mice were 25-30% smaller than +/+ mice from birth till about 5 mos old, no clinically significant differences were observed between these two groups (hematocrit, blood pressure, fasting blood glucose, serum and urine [Na]/[K], and daily food and water consumption). Glucose tolerance test and in vivo resistant to oxidative stress, caused by injection of herbicide paraquat, did not show significant difference between those two groups of mice. Thus, Nedd4 has shown to play an important role in promoting cell growth and differentiation during development.

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Neuron-specific protein family member 1 (*Nsg1*) is a putative MeCP2-regulated gene. *I.J. Delgado[1], M. Yaylaoglu[3], D. Sun Kim[1], R. Amir[1], R. Sierra[1], Z. Yu[1], C. Thaller[3], I. Van den Veyver[1, 2].* 1) Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX; 2) Molecular and Human Genetics, BCM, Houston, TX; 3) Biochemistry and Molecular Biology, BCM, Houston, TX.

Rett syndrome (RTT) is a neurodevelopmental disorder that affects 1/15,000 females. Most RTT patients have mutations in the *MECP2* gene, encoding the methylation-sensitive transcriptional repressor methyl-CpG binding protein 2. Candidate approaches have identified verified MeCP2 targets, such as BDNF. In contrast, expression microarray analysis on brain of RTT mouse models and human patients have been less successful at finding MeCP2 targets; probably because mixed brain regions and/or cell types are interrogated. Furthermore, these studies have not searched for MeCP2 targets during neuronal differentiation. To study the role of MeCP2 during neuronal differentiation and identify target genes using a purer cell population, we performed *in vitro* neuronal differentiation of mutant $Mecp2^{R308/Y}$ and wild-type (WT) mouse embryonic stem (ES) cells using a standard retinoic acid induction protocol. We interrogated gene expression profiles at day 5 of differentiation on total RNA from 3 pooled WT and 3 pooled $Mecp2^{R308/Y}$ ES cell cultures on oligonucleotide arrays and identified 65 upregulated genes and 146 downregulated genes. Real-time quantitative RT-PCR (qPCR) so far confirmed statistically significant changes for 3 genes. We selected neuron specific protein family member 1 (*Nsg1*), a highly-expressed neuron-specific protein involved in receptor recycling at the early endosomes for detailed analysis. A 6-fold upregulation in $Mecp2^{R308/Y}$ differentiating ES cells was found by qPCR. Similar analysis of various mouse brain regions at 5 weeks of age showed a ~2-fold upregulation in cerebral cortex of $Mecp2^{R308/Y}$ mutant mice. RNA in situ hybridization on E14.5 embryos confirmed this upregulation in cortex. These results indicate that *NSG1* expression levels in cortex are regulated by *MECP2* during development and after differentiation. We are currently investigating if *Nsg1* is directly regulated by MeCP2 and how its upregulation when *Mecp2* is mutated contributes to the complex features of RTT.

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Human nasal embryonic luteinizing hormone releasing hormone (NELF), a secreted protein, stimulates GnRH neuron migration. *N. Xu[1,2], L.P. Chorich[1,2], W.C. Xiong[2], L.C. Layman[1,2].* 1) Section of Reproductive Endocrinology, Infertility, & Genetics, Dept OB/GYN; 2) Developmental Neurobiology Program, Institute of Molecular Medicine & Genetics, Medical College of Georgia, Augusta, GA.

Kallmann syndrome (KS) is a disease consisting of the combination of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia or hyposmia. One of the candidate genes for KS is nasal embryonic luteinizing hormone-releasing hormone factor (NELF), which is purported to have a crucial role during the migration process of GnRH neurons from the olfactory placode to the hypothalamus. The cDNA of human NELF was previously cloned in our lab, and the protein does not have any homology to other sequences in the genome and lacks a signal peptide. To test whether NELF is a secreted protein that regulates the migration of GnRH neurons, we made mammalian expression constructs encoding NELF which were tagged with c-myc either at the C or N terminus. Transient transfection of NELF in COS-7 cells and Western blot analysis demonstrated that the NELF protein can be detected in the cultured medium, suggesting that it is secreted. Moreover, immunostaining analysis of transfected COS-7 cells without permeabilization reconfirmed that the NELF protein is distributed on the cell surface. Finally, using conditioned media that contains NELF protein, we demonstrated an increased cell migration of GN11 and NLT cells, but not GT1-7 cells by the Boyden chamber assay. Taken together, these findings indicate that human NELF is a secreted protein that stimulates GnRH neuron migration. The Boyden chamber assay will be a useful in vitro assay to analyze the functional effects of human NELF mutations in patients with IHH. (L.C.L. is supported by NIH grants HD040287 and HD033004; W.C.X. is supported by NIH grants GM063861 and AR048120).

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Discoveries with Computational Analysis of SAGE Data of Mouse Germ-Cell Transcriptome at Different Stages of Spermatogenesis. *T.L. Lee, D. Alba, V. Baxendale, O.M. Rennert, W.Y. Chan.* Lab of Clinical Genomics/NICHD, National Institutes of Health, Bethesda, MD.

Serial analysis of gene expression (SAGE) provides a global analysis platform for profiling mRNA populations present in cells of interest without the constraint of gene selection and the ambiguous nature of data obtained. However, most of reports to date provide data on SAGE analysis and germ cell development are limited to descriptive analysis. Here, we report a series of bioinformatic analysis using recent published SAGE data on the transcriptome of mouse type A spermatogonia (Spga), pachytene spermatocytes (Spcy), and round spermatids (Sptd). Tags with at least a total count of 20 by combing Spga, Spcy and Sptd were examined. Our aim was to identify and discover pathways and their regulators involved at different stages of spermatogenesis. Unsupervised hierarchical clustering based on tag expression and gene ontology analysis were applied to identify genes and biological process over-represented at a particular stage of development. Potential biological networks were then constructed to reveal the link between the gene candidates. The 5 cis-regulatory elements were also examined for common regulators in different functional clusters. Biological pathways related to the three germ cell stages were constructed. A number of key transcription regulators were commonly found along the process, which included NF-B, SP1, AP-1 and Egr1. Taken together, the approach led to the generation of signature networks and promoter modules essential for specific stages in spermatogenesis, which provide information for designing male contraceptive drugs and a better understanding on male infertility.

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TaqMan-based miRNA profiles classify ES and differentiated cells. *C. Chen[1], A.J. Broomer[1], D.A. Ridzon[1], K.J. Guegler[1], W.M. Strauss[2].* 1) Advanced Research & Technology, Applied Biosystems, Foster City, CA 94404, USA; 2) Department of Molecular, Cellular, & Developmental Biology, University of Colorado, Boulder, CO 80309, USA.

TaqMan miRNA assays have been developed using stem-loop primers for reverse transcription (RT) followed by real-time PCR. A total of 252 mouse and human miRNA assays were tested with four different mouse embryonic stem (ES) cell lines and five differentiated embryoid bodies (EBs) and six mouse tissues. Of 238 human miRNAs examined, 215 (90%) were detected in mouse tissues. MicroRNA expression profiles or fingerprints can classify the ES cells, differentiated EBs and adult tissues. MicroRNA expression levels globally increase upon ES cell differentiation. We have identified a number of ES-specific and differentiation-related miRNA candidates that could be used as molecular markers to determine ES cell identity and to monitor its spontaneous differentiation. There exists significant variability in miRNA expression among four ES lines, suggesting that some ES cell cultures may contain a variable portion of spontaneously differentiated cells. Pre-amplification based TaqMan miRNA assays are being developed to detect all miRNAs from a single ES cell. Single-cell miRNA profiles will be invaluable in exploring stem cells and the different types of cells that stem cells can differentiate into. We have also developed TaqMan siRNA assays that can be used to monitor the level of siRNA and to optimize shRNA constructs.

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Genetic modifiers of 22q11DS by expression profiling. *J. Liao, S. Nowotschin, V. Aggarwal, B.E. Morrow.* Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY.

The 22q11.2 deletion syndrome (22q11DS, aka velo-cardio-facial syndrome/DiGeorge syndrome) is a congenital anomaly disorder affecting structures derived from the pharyngeal apparatus including the craniofacial region, thymus gland and heart. Most affected individuals have the same sized 3 Mb deletion, yet the disorder varies in severity. One of the greatest challenges is to determine the basis for its varied expressivity. Haploinsufficiency of *TBX1*, a T-box containing transcription factor, is responsible for the main physical malformations of the syndrome. Stochastic, environmental and genetic factors likely modify the phenotype. Genes in the molecular pathway of *TBX1* may serve as genetic modifiers. The strongest evidence is provided by genetic background effects on the severity of the syndrome in Tbx1 +/- mouse mutants. Heterozygous mice were backcrossed over 10 generations in different genetic backgrounds. We found 45% of Tbx1 +/- mice die when congenic in C57Bl/6 but not in FVB, where they survive in normal Mendelian ratios. One way to identify modifiers is to ascertain whether there is a genetic interaction between co-expressed genes. One particular candidate modifier gene is *Pitx2*, the gene for Rieger syndrome. It is expressed asymmetrically in the anterior heart field. We found that most Tbx1 +/-; Pitx2 +/- mice die at birth with multiple heart defects. Tbx1 and Nkx2.5 synergistically activate the *Pitx2c* enhancer. Another way to identify modifiers is to assess global gene expression changes. We performed Affymetrix expression microarray studies on microdissected pharyngeal arches in wildtype and Tbx1 -/- mouse embryos at multiple developmental stages. *Dlx1*, *Dlx5* and *Lhx8* in the neural crest, *Pax9* and *Gcm2* in the endoderm, *Myf5*, *Gata4*, *Gata6* and *Tbx5* in the mesoderm, comprise a subset of relevant genes identified that have been validated by *in situ* hybridization. Some of them are dependent on the *Tbx1* null mutation and others are changed due to morphology differences. We are working towards identifying a set of strong candidate genes to then test for association in human 22q11DS patients with the same sized deletion.

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Sequence variants of the NANOS3 gene are associated with testis atrophy and a pure sterility phenotype in men.

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Nanos proteins are highly conserved family of RNA-binding proteins and play critical role in germ cells development in many organisms. There are three homologous proteins in human testes: NANOS1, NANOS2 and NANOS3, and they are encoded by autosomal genes. We previously demonstrated that NANOS1 is critical for generation and/or maintenance of spermatogonia stem cells in man. However, the role of NANOS2 and NANOS3 in human germ cell development is still unknown. We screened 124 infertile males with various histological phenotypes of seminiferous tubules (Sertoli Cell Only Syndrome, maturation arrest, hypospermatogenesis or testicular atrophy) for mutations in NANOS2 and NANOS3 genes. We found two types of NANOS3 point mutations in two patients and no mutations in NANOS2 gene. The first mutation was insertion of single amino acid codon, while the second one single nucleotide deletion at +25 position of the intron donor site. These mutations were present only in one allele (the second one being the wild-type) and were absent in 400 fertile men having at least two offspring. The men carrying NANOS3 mutations were azoospermic (lack of germ cells in semen), had partial testicular atrophy, manifesting with thinning of seminiferous tubules and lack of germ cells except spermatogonia. Testes histological phenotype of the patients carrying NANOS3 mutations suggests a potential role of this gene in self renewal of spermatogonia. However, further experiments are needed to find out whether mutations we identified are a cause of male infertility.

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A transcriptional function for SRY in mammalian sex determination. *V.R. Harley[1], L. Ludbrook[1,2], S. Bagheri-Fam[1], H. Sim[1], P. Bernard[1], R. Sekido[3], R. Lovell-Badge[3], K.C. Knower[1,2].* 1) Human Molecular Genetics, Prince Henry's Institute of Medical Research, Clayton, VIC, Australia; 2) Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, Australia; 3) Division of Developmental Genetics, MRC National Institute for Medical Research, London, UK.

The mammalian sex determination pathway is chromosomally controlled as XX gonads develop into ovaries and XY gonads develop into testes. On the Y chromosome is the *SRY* gene which initiates the development of the testis and the male phenotype. In humans, mutations in *SRY* cause male-to-female sex reversal in XY individuals. SRY is a member of the SOX protein family and contains a highly conserved HMG box which binds DNA in a sequence-specific manner *in vitro*, consistent with a role for SRY acting as a transcription factor. Despite 15 years since the discovery of *SRY*, its *in vivo* function has not been established, hampered by the lack of a defined target gene and experimental cell culture systems. Soon after Sry is expressed in the developing XY gonad, *Sox9* is up-regulated. Using a novel reporter assay, we investigated the mechanism by which SRY activates a urogenital ridge specific enhancer element of the *SOX9* gene. The assay revealed facets of SRY action including defects in SRY clinical mutants, post-translational modifications of SRY, subnuclear localisation, and co-activators required to activate the *SOX9* enhancer. Up-regulation of *SOX9* in the testis is a key process of sex determination observed in a wide range of vertebrates, regardless of the presence/absence of *SRY*. This study is the first to establish a cell-based assay for SRY transcriptional function and to demonstrate, at least in humans, that the role of SRY is to up-regulate *SOX9*.

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LHX9, EMX2, FGF9, GATA4, FOG2 AND M33 Appear to be Sex-Determining Genes in the Developing Human Gonad. *H. Ostrer, H. Huang, R. Masch, E. Shapiro.* New York University School of Medicine, New York, NY.

Mouse mutant models of Lhx9 and Emx2 are associated with gonadal agenesis, and mouse mutant models of Fgf9, M33, Fog2 and Gata4 are associated with gonadal dysgnesis in XY animals. To test whether LHX9, EMX2, FGF9, GATA4, FOG2 and M33 function as sex-determing genes in humans, we assessed their expression in developing gonads, using antibodies specific to these genes and to specific cell types. Gonads and genitourinary structures were obtained from human fetuses, 7 to 22 weeks gestational age. Representative sections were stained with antibodies to P450scc, PECAM, smooth muscle actin, SOX9, C-KIT and AMH to identify specific cell types and then with antibodies to LHX9, EMX2, FGF9, GATA4, FOG2 and M33. At 7 weeks, prior to testis determination, cytoplasmic expression was observed for LHX9, FGF9 and M33, membranous expression was observed for EMX2, and cytoplasmic and nuclear expression was observed for GATA4 and FOG2. After 7 weeks, expression was observed for all of the genes in multiple cell types in both testis and ovary. In the developing testis, expression was observed in Sertoli cells for all of the genes. These observations suggest that these genes are testis-determining in humans and provide criteria for identifying new candidate genes for testis development. These genes should be expressed in undifferentiated gonadal ridges and should continue to be expressed in Sertoli cells after testis determination has taken place - similar to what we have observed previously for SRY, SOX9, DAX1, WT1, and SF1. Nuclear expression does not appear to be required as the proteins can apparently be shuttled into the nucleus, if that is their apparent site of action. Expression in both developing testis and ovary following gonadal determination suggests that these genes play other roles in gonadal development.

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Functional identification of candidate genes using hematopoietic stem cell (HSC) expression data and genetic screening. *E. Mendenhall[1], C. Eckfeldt[1], C. Verfaillie[1], S. Ekker[2].* 1) Stem Cell Institute, University of Minnesota, Minneapolis, MN; 2) Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN, USA.

Introduction: The genetic mechanisms that govern HSCs are poorly understood. To delineate these mechanisms we compared the expressed gene profile of cells enriched for HSC with cells enriched for hematopoietic progenitors, from bone marrow (BM) and umbilical cord blood (UCB). Subsequently, we performed gain-of-function (GOF) and loss-of-function (LOF) genetic screens in zebrafish to rapidly identify genes with a functional role in hematopoiesis. **Results:** 277 genes were differentially expressed in both UCB and BM HSC vs. HPC. Currently, 73/277 human genes that had a putative zebrafish orthologue and had no known role in HSC biology were identified as candidate genes. For LOF studies, we designed antisense morpholino oligos (MOs) against the 73 genes and injected into zebrafish embryos. 14/73 MOs lead to observable defects in hematopoiesis. To quantify this we used Q-RT-PCR for hemoglobin (Hb) and L-plastin. For 5/7 MOs, a >2 fold reduction in Hb and L-plastin mRNA was detected. Specificity of antisense targeting was confirmed using a second MO of independent sequence for 4/5 genes. These candidate genes include SPRY1, FOXM1, SNX5, PRKCB, and JJAZ1. We also performed a GOF screen for 7 genes that did not have an orthologue in zebrafish by cDNA overexpression of the human gene in zebrafish. 1/7 genes showed a decrease in blood formation; hence direct overexpression of human genes in zebrafish can be used to identify candidate genes. Mammalian studies are ongoing for many of these genes. We overexpressed SPRY1 cDNA in human UCB cells via lentiviral transduction and transplanted them in NOD/SCID mice. The SPRY1 overexpressing cells failed to contribute to hematopoiesis compared to mock-transduced cells (0.06% vs. 9%), confirming our zebrafish data indicating a role for SPRY1 in hematopoiesis. **Conclusions:** We show that the combination of gene expression analysis and LOF and GOF screens in zebrafish offers a quick and effective way to identify candidate genes critical to developmental processes.

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Dual expression domains of *Tbx1* in the otic vesicle and periotic mesenchyme are required for normal patterning of the ear. *J.S. Arnold[1], E.M. Braunstein[1], T. Ohyama[2], J.M. Hebert[1], E.B. Crenshaw[3], A.K. Groves[2], M.C. Brown[4], J. Adams[4], B.E. Morrow[1].* 1) Albert Einstein Col Medicine, Bronx, NY; 2) House Ear Institute, Los Angeles, CA; 3) The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Massachusetts Eye and Ear Infirmary, Boston, MA.

Tbx1, the gene responsible for 22q11 deletion syndrome, is required for ear development. The outer and middle and inner ear fail to form in Tbx1 -/- mutants. In contrast, the cochleovestibular ganglion (CVG), is duplicated in Tbx1 -/- embryos, suggesting that *Tbx1* both induces sensory organ formation and suppresses neural cell fate determination. *Tbx1* is expressed in the otic vesicle and the surrounding periotic mesenchyme. Epithelial-mesenchymal interactions between the two tissues are necessary for normal patterning of the ear. To determine the role of *Tbx1* in each cell type we generated a conditional allele of the gene. Foxg1-Cre was used to drive otic vesicle specific ablation of *Tbx1*. Inactivation of *Tbx1* in the otic vesicle resulted in hypoplasia of the inner ear sensory structures and duplication of the CVG. The Foxg1-Cre strain also mediates *Tbx1* ablation in the first pharyngeal pouch endoderm, which forms the auditory tube. In the conditional mutants the first pouch did not extend towards the external auditory meatus preventing the outer and middle ear from forming. To assess the specific role of *Tbx1* in inner ear development, we used the Pax2-Cre strain to ablate the gene in the otic vesicle while maintaining the expression domain in the first pouch. Mutant mice had normal outer and middle ears but had the same inner ear defects as *Tbx1* null mice, underscoring its importance in the otic vesicle. In a complementary experiment, we used Brn4-Cre mice to ablate *Tbx1* in the periotic mesenchyme. While mutant mice showed normal outer and middle ear development, the cochlea had significant malformations. These results show that *Tbx1* in the periotic mesenchyme is responsible for cochlear formation, as mutants had cochlear hypoplasia but normal capsule and middle ear bones. Overall, our data demonstrate the essential role of *Tbx1* in epithelial-mesenchymal interactions responsible for patterning the ear.

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Genetics of Vertebrate Caudal Limb Field Specification. *J.A. Lehoczky[1], D. Beier[2], W.-W. Cai[3], J. Moran[2], J.W. Innis[1].* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Division of Genetics, Harvard Medical School,Brigham and Women's Hospital, Boston, MA; 3) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Studies into the genetics of vertebrate limb initiation and patterning have identified tissues and specific genes associated with the process of limb bud outgrowth. However, the mechanisms responsible for establishing limb fields prior to the expression of outgrowth genes such as TBX4/5, Wnts, and FGFs, in specific A/P locations are poorly understood. We have characterized a new, spontaneous mouse mutant, Polypodia (Ppd), which we discovered here at the University of Michigan. This mutant exhibits ectopic caudal limbs and we hypothesize that Ppd activates a limb and caudal appendage-inducing activity. Humans with ectopic lower limbs with or without pelvic anomalies, but not spine duplication, have been described extensively in the literature. Ppd is autosomal dominant with 20% penetrance on the C3H background and has a normal karyotype. We crossed Ppd to C3H and genotyped 402 SNP markers and numerous STR markers in each of 45 affected offspring. Current SNP haplotype and STR allele data has allowed us to exclude are large amount of the genome including the relevant chromosomal regions of Pitx1, Tbx4, Tbx5, Fgf8, Fgf10, the Disorganization locus, Raldh1, Raldh2, Raldh3, Cyp26-a1, -b1 and -c1, and the RAR/RXR genes. To further our genetic mapping we have initiated a new cross to CAST/Ei. We have also completed a comparison of the genomes of Ppd animals with controls using Array CGH. In these experiments, genomic DNA from the original Ppd male was compared to CD-1 DNA, and a generation 4, Ppd female on the C3H background was compared to a wild-type C3H female in arrays with 19,200 BACs across the entire mouse genome. No large scale (>100 kb) deletion or duplication was observed. Ppd is a novel, new mouse mutant without any counterpart that will promote new knowledge into the genetic constituents that define the position and number of caudal limb fields.

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A novel spontaneous mouse mutation that causes congenital dipypus (caudal duplication) and sex cord tumors. *K. Tatsumi[1], A. Lindgren[2], S. Eames[1], A. Wolfe[2], K. Millen[1].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Pediatrics, University of Chicago, Chicago, IL.

Recently, a spontaneous mutation arose in our mouse colony resembling congenital dipygus, an extremely rare human birth defect characterized by supernumerary lower limbs and/or pelvis. Affected mice have a variable phenotype with supernumerary limbs caudal to the normal hindlimbs with fur, bone and vasculature. Radiographs and skeletal staining reveal that the ectopic legs are usually attached to a mirror-image duplication of pelvic structures, located directly posterior to the normal pelvis along a slightly elongated spine. This developmental abnormality is not simply a posterior axial duplication since the tail is not concurrently duplicated. Several internal organ abnormalities have also been noted. In particular, all affected mice also have had sex cord tumors - granulosa cell tumors in females and Sertoli cell tumors in males. Extensive breeding analysis has determined that this is a dominant mutation with low penetrance. The homozygous phenotype is apparently embryonic lethal. Mapping studies are underway. Affected mice have phenotypes reminiscent of the Disorganization (Ds) mutation. Ds is a dominant, gain of function mutation with low penetrance that maps to mouse chromosome 14. Ds mutants have disorganized body plans and display a range of developmental defects so great that no two mice show the same phenotype. Ds mice never develop cancer. In contrast, our new mutant has a generally consistent phenotype which includes tumors, suggesting that this new mutation represents a novel mouse locus. Since the observed skeletal abnormalities present a challenge to current theories of body axis and limb formation, analysis of this new mutant is certain to provide new insights into vertebrate patterning.

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Mouse models of Down syndrome trisomic for all human chromosome 21 syntenic regions. *Y. Yu[1,2], Z. Li[1], P. Szurek[1], A. Pao[1], S. Rivera[3], S. Matsui[1], J. Roder[4], A. Bradley[5].* 1) Genetics Program, Roswell Park Cancer Inst, Buffalo, NY; 2) New York State Center of Excellence in Bioinformatics & Life Sciences; 3) Baylor College of Medicine, Houston, TX; 4) Mount Sinai Hospital Research Inst, Toronto, Canada; 5) Sanger Institute, Hinxton, UK.

Approximately 95 percent of Down syndrome (DS) patients carry an extra copy of the entire human chromosome 21, which is the leading genetic cause of mental retardation. Genes in different regions of human chromosome 21 are associated with a broad range of cellular pathways or processes. Triplications of key genes in different regions may perturb these events, leading to mental retardation, which is consistent with data from studies of segmental trisomy 21. Since the identities of many of the key genes in these regions are unknown, an ideal mouse model should be segmentally trisomic for all of the human chromosome 21 syntenic regions, which are located on mouse chromosomes 10, 16, and 17. To develop this model, we have generated mice carrying a 2.3-Mb trisomy for the entire syntenic region on mouse chromosome 10 by using chromosome engineering. Furthermore, our preliminary result suggests that a 1.2-Mb duplication that spans the entire syntenic region on mouse chromosome 17 may have been established in ES cells. Finally, we have targeted a loxP to the 1st endpoint in ES cells in order to generate a 22.9-Mb duplication in the syntenic region of mouse chromosome 16. Chimeras have been developed with these cells and the targeting vector for the 2nd endpoint has been generated. The desired model derived from this effort will be more complete than Ts65Dn, which is trisomic for about 56 percent of the orthologous genes of human chromosome 21. We will characterize the phenotypes of the new mouse models that may be related to DS-associated mental retardation by using behavioral, neurophysiological, and neuropathological approaches. These studies will lay the foundation for achieving our long-term goal of unraveling the molecular mechanism of DS-associated mental retardation and may lead to new opportunities for developing effective therapeutic interventions for the clinical manifestations of DS, including mental retardation.

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Phenotypic, genetic and developmental characterization of *cpo1*, a recessive ENU-induced mouse model of cleft palate. *B.C. Bjork[1], A.R. Vieira[2], S. Faust[3], S.A. Camper[3], J.C. Murray[2], D.R. Beier[1].* 1) Div Genetics, Brigham & Women's Hospital, Boston, MA; 2) Dept. Pediatrics, Univ. of Iowa, Iowa City, IA; 3) Depts. Human Genetics and Internal Medicine, Univ. of Michigan, Ann Arbor, MI.

Our ENU mutagenesis screen generates a large spectrum of recessive phenotypes similar to human congenital disorders, including several craniofacial defects. Clefting occurs frequently in mice, but those with isolated clefts are ideal models of human non-syndromic cleft lip/palate (NSCL/P). The *cleft palate only 1* (*cpo1*) mutation is an excellent such model.

The *cpo1* mutant phenotype is due to a mutation that affects splicing efficiency of the zinc finger transcription factor *Prdm16* on chr 4 (Hs. 1p36) and is likely hypomorphic. Mutant mice have a specific defect in palatal shelf elevation. Preliminary palate explant culture shows that *cpo1* palate shelves elevate and meet at the midline upon removal of the mandible and tongue, suggesting that a physical obstruction is primarily responsible for the clefting phenotype. *Prdm16* expression in developing craniofacial structures is consistent with it playing a critical role during palatogenesis.

We identified three potential etiologic missense mutations in a screen of 200 NSCL/P cases from Iowa and the Philippines; these are not observed in 200 controls. One of these, a G819S mutation, was subsequently identified in 54/1064 individuals in the CEPH Diversity panel but a modifying role remains a possibility. The *Prdm16* paralog *Evi-1* is known to act in the *Tgf* signaling pathway. Therefore, we are utilizing *in vitro* analysis of a *TGF*?responsive luciferase reporter to assay the effect of *PRDM16* on *TGF* signaling and any potential functional consequence of the three *PRDM16* missense mutations.

We continue to refine the *Prdm16* expression pattern and have begun to look for misexpression of craniofacial markers in *cpo1* mutants to place *Prdm16* in a genetic pathway. Finally, in addition to gene targeting, we are using RNAi transgenesis to rapidly "knock down" *Prdm16* in mouse embryos and validate the etiology of the *cpo1* mutation.

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Gene Expression Profiling of Cardiac Angiogenesis Using a Canine Model. *C. Gregory, T. Masters, A. Fokin, F. Robicsek.* Heineman Medical Research Laboratory, Carolinas Heart Institute, 1001 Blythe Blvd., Charlotte, NC.

Coronary heart disease remains the leading cause of morbidity and mortality in the United States. Despite the advances in myocardial revascularization, a large group of patients are not candidates for current surgical or cardiological interventions to restore blood flow. A possible treatment for these patients may be neovascularization via angiogenesis. Numerous studies have implicated various growth factors to be involved in this process. However, the precise sequence of gene expression involved in initiation and maintenance of angiogenesis has not been delineated for clinical application. Using a canine model of myocardial ischemia, angiogenesis was amplified by an AV fistula (distal occluded circumflex to adjacent cardiac vein). Tissue from collateral vessels was taken, on day 8 after the AV fistula, from 5 experimentals and 4 controls. Gene expression profiling was accomplished using the Affymetrix GeneChip Canine Genome Array. Using ArrayAssist and PathwayAssist, ~3,900 probe sets were significantly up or down regulated. Further refinement found 168 probe sets with differential expression log scale of -1 and 109 probe sets with +1 and a p value 0.05. Annotation of the probe sets, revealed an array of upregulated candidate genes involved in cell proliferation, differentiation, motility, and regulation of signal transduction. Some of the genes included in this group are LECT2, APBA1, RAD52, ANGPT1, MBL1, IMPD2, UCK2 and KHDRB. Hierarchical cluster analysis showed 3 distinct groups (53, 20, 8 probe sets) of temporal patterns from the animals that demonstrated development of angiogenesis. In the cluster of 53 probes, 33 were unknown status and 20 were annotated. The 20 annotated genes of the cluster were found to be involved with cell proliferation, differentiation, and regulation of signal transduction. In conclusion, this study has provided a snapshot of gene expression patterns at day 8 in the process of angiogenesis. This data enables further refinement of the genes that will be critical to initiate and maintain neovasuclarization.

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DNA methylation and gene expression at the candidate locus for transient neonatal diabetes mellitus is tissue specific during fetal and postnatal development. Implication for pancreatic cells function. *G. Marcelin[1], A. Nicole[1,2], C. Diatloff -Zito[1,3].* 1) Inserm U383, Groupe Hospitalier Necker-Enfants Malades 149-161 rue de Sèvres 75743 Paris Cedex 15 France; 2) Faculté de Médecine Paris 5; 3) CNRS.

Epigenetic alterations are involved in a number of human diseases including developmental abnormalities, cancer and endocrine diseases. Defects within the imprinted region of chromosome 6q24.2 have been implicated in transient neonatal diabetes mellitus (TNDM), a developmental disease of insulin production characterized by hyperglycemia at birth, absence or low levels of insulin. About 65% of TNDM patients will have type 2 diabetes at adolescence. Loss of methylation at CpG island within the (differentially methylated region) DMR was observed in most patients studied with a normal karyotype. The DMR of *ZAC1/HYMAI* may potentially regulate expression of imprinted genes within the domain, and epigenetic or genetic mutations of this region may result in TNDM by affecting expression of *ZAC1/HYMAI* and new candidates. In order to test this hypothesis and approach mechanisms involved in TNDM pathogenesis, genomic DNA methylation coupling to gene transcription of *Zac1* and other epigenetically regulated regions within the locus,were determined in mice pancreas and heart in vivo. The 10A2 mouse locus syntenic to the human 6q24.2 are highly conserved in the two species. Data from mice heart and pancreas tissues at different fetal ages (E12 to birth) and postnatal (4 weeks to one year), as well as from pancreatic islets, ex vivo and MIN-6 culture cells, in vitro are reported. Evidences showing that spatio-temporal methylation dynamic and transcription is tissue specific are provided. An active epigenetic process of the 6q24.2/10A2 locus related to pancreas physiological activity is suggested.

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Gene expression microarray analysis of mouse orofacial development. *R. Spritz[1], W. Feng[2], T. Pfang[3], L. Hunter[1,3], M. Geraci[1,4], T. Williams[1,2].* 1) Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Aurora, CO; 2) Dept Craniofacal Biol, Univ Colorado Hlth Sci Ctr, Aurora, CO; 3) Dept Pharmacol, Univ Colorado Hlth Sci Ctr, Aurora, CO; 4) Dept Medicine, Univ Colorado Hlth Sci Ctr, Aurora, CO.

Orofacial clefts are among the most common of all birth defects, cleft lip cleft palate (CL/P) occurring with a frequency of ~1/500 to 1/2500 in different populations. Despite much effort, few CL/P genes have been identified in humans. We are using the mouse as a model system to identify potential candidate genes for human orofacial clefts. In vertebrates the face develops mainly from 3 bilaterally paired prominences: maxillary prominences (MxP), frontal nasal prominences (FNP), and mandibular prominences (MdP). In mouse most orofacial morphogenesis takes place from embryonic day (ED) 10.5 to 12.5. We have dissected the MxP, FNP, and MdP separately from wild-type (C57BL6/J) ED10.5 to ED12.5 mouse embryos at 0.5 ED intervals, extracted RNA, and carried out microarray analyses using Affymetrix mouse genome 430 2.0 arrays, which interrogate expression of ~39,000 transcripts. All dissections and microarray assays have been carried out in 7-fold replicate to provide high data reliability. Data reproducibility has been excellent, and expression of specific indicator genes has been used to monitor purity of dissected tissues. Data mining has yielded lists of genes whose expression is either tissue-specific or that vary in a time-dependent manner during orofacial development. We have specifically analyzed developmental expression patterns of a number of genes that have been implicated in human syndromic and non-syndromic orofacial clefts. We are also carrying out analogous parallel dissections and analyses of two lines of mutant mice, one with mid-facial clefting and one with bilateral cleft lip cleft palate, to provide important comparisons between normal and abnormal orofacial developmental patterns. We anticipate that these studies will highlight new candidate genes, genetic networks, and developmental pathways that may be involved the pathogenesis of human orofacial clefts.

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Group 13 HOX proteins interact with the MH2 domain of R-Smads and modulate Smad transcriptional activation functions independent of HOX DNA binding capability. *J.W. Innis[1,2], T.M. Williams[1], M.E. Williams[1], J.H. Heaton[1], T.D. Gelehrter[1].* 1) Department of Human Genetics, Univ. Michigan, Ann Arbor, MI; 2) Department of Pediatrics, Univ. of Michigan, Ann Arbor, MI.

Interactions with cofactors provide a means by which HOX proteins exert specificity. To identify candidate protein interactors of HOXA13 we created and screened an E11.5-E12.5, distal limb bud yeast two-hybrid prey library. Among the interactors we isolated the BMP-signaling effector Smad5, which interacted with the paralogous HOXD13 but not with HOXA11 or HOXA9, revealing unique interaction capabilities of the AbdB-like HOX proteins. Using deletion mutants, we determined that the MH2 domain of Smad5 is necessary for HOXA13 interaction. This is the first report demonstrating an interaction between HOX proteins and the MH2 domain of Smad proteins. HOXA13 and HOXD13 also bind to other BMP and TGF-/Activin-regulated Smad proteins including Smad1 and Smad2, but not Smad4. Furthermore, HOXD13 could be co-immunoprecipitated with Smad1 from cells. Expression of HOXA13, HOXD13 or a HOXD13 homeodomain mutant (HOXD13IQN>AAA) antagonized TGF--stimulated transcriptional activation of the pAdtrack-3TP-Lux reporter vector in Mv1Lu cells as well as the Smad3/Smad4-activated pTRS6-E1b promoter in Hep3B cells. Finally, using mammalian one-hybrid we show that transcriptional activation by a GAL4/Smad3-carboxy terminus fusion protein is specifically inhibited by HOXA13. Our results identify a new cofactor for HOX group 13 proteins and suggest that HOX proteins may modulate Smad-mediated transcriptional activity through protein-protein interactions without the requirement for HOX monomeric DNA binding capability.

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Expression of Pax3 and Pax7 during mouse embryogenesis: comparison to MyoD and Myogenein. *J. Horst[1], D. Horst[2], C. Sergi[2], H. Jürgens[3], E. Vorobyov[1].* 1) Institut für Humangenetik, Universitätsklinikum Münster, Germany; 2) Institut für Pathologie, Medizinische Universität Innsbruck, Austria; 3) Klinik und Poliklinik für pädiatrische Hämatologie/Onkologie, Universitätsklinikum Münster, Germany.

Pax3 and Pax7 encode homologous transcription factors the expression of which is known to be required for the dermomyotome formation in the developing trunk. However, it is not yet clear whether these genes are required for myogenic cell specification in the head and for the post-somitic myogensis per se. In part, this uncertainty is due to the scarce information about their normal time course and pattern of expression. Here, we present a systematic in situ analysis of spatiotemporal characteristics of Pax3 and Pax7 gene expression in comparison to that of MyoD and Myogenin, with special attention to the developing muscles of the head. The expressed proteins were immunohistochemically detected on adjacent sections of embryos at stages E11-15 and postnatal muscles. The observed patterns of expression suggest that Pax3 is not involved in myogenesis in the head, and its post-somitic expression in the trunk and limb muscles is repressed after stage E13. In contrast, Pax7 expression is shared among all striated muscles and exhibits a uniform pattern that can be explained by an asymmetric cell division. In the developing muscles of the head Pax7 is activated after MyoD and as such cannot be considered as a myogenic inducer in the head. These data reveal that Pax3 and Pax7 exhibit distinct spatiotemporal expression modes in myogenic precursors during embryogenesis.

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Gene Expression Profiling of Human Pharyngeal Arch 2. *J. Cai[1], D. Ash[1], L. Kotch[1], E.W. Jabs[1], Y. Korshunova[2], R. Tidwell[2], D. Messina[2], J. Winston[2], M. Lovett[2], T. Attié-Bitach[3], J. Augé[3], G. Mattei[3], H. Etchevers[3], M. Vekemans[3].*
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Craniofacial abnormalities are one of the most common birth defects in humans, yet little is known about the genes that control these important development processes. To identify relevant genes, the COGENE consortium is profiling gene expression to study normal human craniofacial development. Using microdissected, normal human craniofacial structures, we constructed 12 SAGE (Serial Analysis of Gene Expression) libraries and sequenced 606,532 tags. We also performed Affymetrix microarray analysis on 25 craniofacial targets. We then analyzed expression profiles of pharyngeal arch 2 (PA2) that forms the stapedial artery, muscles of facial expression, the posterior belly of the digastric, stylohyoid and stapedius muscles, stapes, styloid process, stylohyoid ligament and facial nerve VII. Comparison between SAGE libraries revealed that 27 tags were upregulated and 50 tags were downregulated (p0.01), whereas microarray analysis revealed that 11 and 23 genes were upregulated and downregulated (p0.01), respectively, from 4th to 5th week PA2. Protein biosynthesis was overrepresented in the differentially expressed genes determined by SAGE, and cell communication process was overrepresented in the differentially expressed genes determined by microarray analysis. By comparing SAGE and microarray data, genes that are enriched in PA2 were identified, many of which were previously unknown to be expressed in PA2. Whole mount in situ hybridization was performed on mouse embryos at corresponding stages to study the conservation of expression patterns observed in humans. For example, the Wbscr1 gene, whose human homolog is in the Williams-Beuren syndrome critical region, was expressed both in mouse PA2 precursor cells at GD8.5 and in PA2 at GD9.5, and by GD10.5, it was highly expressed in PA2, especially in the posterior half. This data should provide useful information of gene expression in normal PA2 development, and help in identifying pathways and genes that contribute to common pharyngeal arch defects.

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Pretranslational Upregulation of COL1A2 is not Responsible for the Type I Collagen Glomerulopathy in COL1A2 Deficient Mice. *A.C. Brodeur, B.J. Pfeiffer, J.R. Gentry, D.A. Wirth, A.M. Roberts-Pilgrim, C.L. Franklin, L.W. Reneker, C.L. Phillips.* Univ of Missouri-Columbia, Columbia, MO.

We recently identified a novel type I collagen glomerulopathy in COL1A2 deficient mice (homozygous for a functional null pro2(I) collagen gene), which synthesize exclusively homotrimeric type I collagen, $1(I)_3$. The role of the homotrimer is currently unknown. Examination of COL1A2 deficient mice revealed type I collagen deposition in the mesangium of glomeruli in both homozygous and heterozygous mice. Using *in vivo* and *in vitro* approaches, we investigated the pathological and mechanistic significance of the COL1A2 deficient glomerulopathy. To evaluate pathologic significance we used two histologic scoring systems to study the presence and severity of the glomerulopathy within individual animals. We demonstrate that the glomerulopathy initiates postnatally following glomerular maturation and is progressive with a gene dosage effect. To determine the role of type I collagen synthesis in glomerular deposition we examined steady state 1(I) and 2(I) collagen mRNA levels across all genotypes from 1 day to 1 month of age by *in situ* hybridization (ISH). ISH analyses showed similar temporal 1(I) and 2(I) collagen mRNA levels in glomeruli of all three genotypes. To confirm the ISH findings, we performed quantitative real-time PCR on total mRNA isolates from age-matched kidneys of each genotype. Real-time data confirms that there is no significant difference in temporal 1(I) and 2(I) mRNA. Therefore, we suggest glomerular deposition seen in the type I collagen glomerulopathy is not due to pretranslational mechanisms. Degradation of type I collagen within the glomerulus is primarily regulated by matrix metalloproteinases (MMPs). Previous studies have identified the presence of MMPs 1, 2, 3, 9, 13, and 14 in the kidney. MMPs 1, 2, 3, 8, 9 and 13 are known to cleave type I collagen and its molecular components. Preliminary studies using *in vitro* enzymatic analysis suggest that there is differential cleavage of hetero- and homotrimeric type I collagen by specific MMPs. Further studies will elucidate the involvement of individual MMPs.

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Identification of Genes Regulated by the Nuclear Receptor *Nr2e3* to Direct Photoreceptor Development. *N. Haider.* Department of Genetics, Cell Biology and Anatomy, Univ Nebraska Medical Ctr, Omaha, NE.

Mutations in *Nr2e3* cause Enhanced S-Cone Syndrome in humans and are responsible for the *rd7* phenotype in mice. We used the *rd7* mouse as a model to study the molecular mechanisms underlying human retinal degeneration. Our previous studies revealed that *Nr2e3* is important for the development and specification of rod and cone photoreceptors; its absence disrupts retinal integrity and photoreceptor function. *rd7* mice exhibit an abnormal increase in cone photoreceptor population which is due to continued cone cell proliferation beyond the time when photoreceptors normally exit the cell cycle. In the present study, we use the *rd7* mouse to identify molecules that are regulated by *Nr2e3* to direct proper development of photoreceptors. Real-time PCR analysis was performed on over 100 candidate transcriptional targets of *Nr2e3*. Misregulated genes were evaluated for the presence of upstream response elements that NR2E3 could bind. These elements were tested by *in vitro* electrophoretic mobility shift assay to determine which genes are direct targets of NR2E3 . In addition, differential expression of proteins was evaluated in *rd7* mice at critical times during photoreceptor development. Differentially expressed proteins were identified and also evaluated for the presence of upstream regulatory sites to which NR2E3 may bind. Our studies demonstrate that *Nr2e3* is a dual regulator affecting the processes of proliferation and differentiation during development as well as photoreceptor function in the adult retina.

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Opposite effect of genetic background in acoustic startle response of fragile X knockout mice. *F. Kooy[1], R. D'Hooge[2], E. Fransen[1], P. De Deyn[2], V. Errijgers[1].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Department of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium.

In this study we have further analyzed the acoustic startle response (ASR) in fragile X knockout mice bred in different genetic backgrounds, including C57BL/6J, (C57BL/6J x 129P2/OlaHsd) F1 and F2 intercross mice. The fragile X knockout mouse lacks Fmrp and shows abnormalities comparable with the symptoms observed in human fragile X patients, including mild to severe learning difficulties, macroorchidism and behavioural alterations. The fragile X knockout mouse model is a valid animal model to study the human disorder.

The ASR can be used as a behavioral tool to assess the neuronal basis of behavior. All mice tested showed an increasing startle response in relation to increasing stimulus intensity. We found differences in ASR between knockouts and controls dependent on genetic background and stimulus intensity. Fragile X knockout mice bred in a C57BL/6J background showed an increase in ASR in response to the lowest stimulus of 90 dB compared to control mice and a decrease in ASR in response to the highest stimulus of 110 dB. Remarkably we found an opposite ASR between F1 and F2 mice. Knockouts of the F1 generation showed a decreased ASR in response to each stimulus intensity used compared to controls, whereas knockouts of the F2 generation showed an increased ASR. Since both F1 and F2 mice consist each of 50% of genetic material from either parental strain, these results show for the first time that the different distribution of the genetic material from the parental strains may influence the fragile X knockout phenotype in the ASR. This suggests that modifier genes may strongly influence the ASR in fragile X syndrome.

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Non-random genetic abnormalities lead to non-random epigenetic changes in leukemia. *S. Rossetti[1], C. Stanciu[1], A.T. Hoogeveen[2], N. Sacchi[1].* 1) Dept Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) Erasmus Medical Center, Rotterdam, The Netherlands.

Non-random leukemia chromosomal abnormalities are a hallmark of distinct leukemia subtypes. Evidence is accumulating that hypermethylation of CpG islands is also non-random in leukemia. We hypothesize that there is a mechanistic link between non-random chromosomal abnormalities involving genes encoding chromatin regulators and epigenetic changes at specific target genes. To test this hypothesis we used as a model AML1-MTG16, a fusion protein produced in t(16;21)-positive leukemia. This protein retains the DNA binding domain of AML1, a transcription factor crucial for normal hematopoiesis, and most of the functional domains of MTG16, encoding a transcriptional corepressor able to recruit histone deacetylase (HDAC) activity. For this reason, AML1-MTG16 is expected to aberrantly regulate AML1 target genes by inducing repressive epigenetic modifications. By integrating gene expression analysis and bioinformatics we identified several aberrantly regulated AML1-target genes in AML1-MTG16-positive cells. By chromatin immunoprecipitation we proved that the AML1-consesus sequence present in the promoter regions of two downregulated genes were directly bound by AML1-MTG16. Noteworthy, these promoter regions were associated with repressive chromatin changes, such as histone H4 deacetylation and aberrant DNA hypermethylation. Our results indicate that integrated approaches can efficiently identify the links between non-random chromosomal abnormalities and non-random epigenetic changes in leukemia.

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Features of imprinting signature at the 6q24.2 locus for transient neonatal diabetes mellitus. *C. Diatloff-Zito[1,2], A. Nicole[1,3], G. Marcelin[1], JJ. Robert[4].* 1) INSERM U383, Hopital Necker Enfants Malades, Paris, France; 2) CNRS, Paris 16; 3) Faculté de Médecine Paris 5; 4) Fédération de Pédiatrie.

Loss of genomic imprinting is involved in a number of developmental abnormalities, neuro behavioral problems and cancer. ZAC1 and HYMAI are imprinted genes expressed from the paternal allele of chromosome 6q24.2 within a region known to harbor a tumor suppressor gene activity and identified as the candidate locus for transient neonatal diabetes (TND). Imprinting defect in the region occurs in TND. A loss of maternal methylation at CpG island within the differentially methylated region (DMR) was observed in most patients studied (61%) of our series with a normal karyotype. The DMR at the 5end of ZAC1 and HYMAI genes may regulate expression of imprinted genes within the domain and epigenetic or genetic mutations of this region may result in TND by affecting expression of ZAC1 or HYMAI in pancreas or the pituitary. Methylation analysis in patients and control individuals shows that the DMR is not homogeneously methylated with discrete tissue specific local pattern supporting the idea of bipartite epigenetic regulation process. Alterations at chromatin level may cause the disease in 23% of the TND patients with no other defect identified. Inter-individual variability in control DNA from blood and pancreas samples, is suggestive of epigenetic polymorphism that may potentially underlie local genomic plasticity.

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Submicroscopic chromosomal rearrangements in Beckwith-Wiedeman patients. *M.P. Recalcati[1], S. Russo[1], P. Finelli[1,2], S. Ferraiuolo[1], D. Giardino[1], F. Cavalleri[1], F. Cogliati[1], M.G. Tibiletti[3], L. Zoccante[4], M.T. Bonati[5], L. Larizza[1,6].* 1) Lab Medical Genetics, Istituto Auxologico Italiano, Milano; 2) Dipartimento di Biologia e Genetica, Università di Milano, Milano; 3) Lab di Anatomia Patologica, Ospedale di Circolo, Varese; 4) Servizio Autonomo, N.P.I., Policlinico G. Rossi, Verona; 5) Genetica Medica, Istituto Auxologico Italiano, Milano; 6) Genetica Medica, Dipartimento di Medicina, Chirurgia e Odontoiatria, Università di Milano.

Beckwith-Wiedemann syndrome (BWS; MIM 130650) is a disorder of growth regulation, characterized by prenatal and postnatal overgrowth, macroglossia, anterior abdominal wall defects and tumor predisposition. The etiology involves genetic and epigenetic alterations affecting a genomic region of approximately one megabase on human chromosome 11p15, which is organised into two imprinted domains under the control of DMR1, 4Kb distal to 5H19, and DMR2 in KVLQT1 IVS10. Only 2% of BWS patients were found to carry cytogenetic abnormalities such as chromosome 11 duplications and translocations. 60 BWS patients was investigated for the most common pathogenetic mechanisms: 11p15 mosaic paternal UPD and defects in methylation pattern of the imprinted H19 and LIT1 genes. Microsatellite segregation analysis, carried out to search 11patUPD, showed in two patients a profile compatible with the presence of subchromosomal duplications. FISH analysis confirmed that one case, trisomic for D11S318 and showing both paternal methylated H19 alleles, carries a de novo dup(11)(p15.5p15.5), likely resulting from unequal recombination at meiosis I in paternal gametogenesis. BAC FISH characterization evidences the breakage separates the two IC domains controlling the BWS region. The second patient was found to carry a der(21)t(11;21)(11p15.4;q22.3)pat originated from missegregation of a cryptic paternal translocation. An additional patient with Wolf syndrome and unusual height was shown to carry a der(4)t(4;11)(p16.3;p15.4)pat characterised by FISH analysis.The latter case with the interstitial direct duplication might provide a tool to investigate the role of each BWS IC in the aetiopathogenesis of the syndrome.

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Complementation test indicates that an IAP transposon insertion at Wnt9b contributes to multifactorial cleft lip and palate in the mouse model. *D.M. Juriloff[1], M.J. Harris[1], T.J. Carroll[2], A.C. Lidral[3].* 1) Dept Medical Genetics, U British Columbia, Vancouver, BC, Canada; 2) Dept Internal Medicine, UT Southwestern, Dallas, TX; 3) Dept Orthodontics, U Iowa, Iowa City, IA.

The genes involved in the genetically complex etiology of human nonsyndromic cleft lip and palate (CLP) have not been identified. The A/WySn mouse strain is a good model; 20 percent of fetuses or neonates have nonsyndromic CLP. Previous work showed that the etiology of the A/WySn defect is digenic - the joint effect of the clf1 recessive gene on Chr11 and the clf2 semidominant gene on Chr13, plus a strong A/WySn maternal effect. Previous extensive work to identify the clf1 mutation excluded sequence differences in the most plausible candidate genes; comparison with recent ancestral sequence pointed to an IAP transposon insertion 6.6 kb from the 3' end of the Wnt9b gene in A/WySn as being the clf1 mutation. Recent gene knockout studies in mice found that about 50 percent of Wnt9b null embryos have CLP as part of a syndrome of defects (Carroll & Lidral in prep). In the present study a complementation test was done by crossing Wnt9b+/- males with A/WySn females (a/a at Wnt9b). 83 F1 fetuses were obtained, examined and genotyped by PCR. The ratio of a/+ to a/- was Mendelian overall. There were 13 CLP fetuses, and all were a/-, indicating noncomplementation between the A/WySn allele at Wnt9b and the null allele, and supporting the interpretation that the IAP interferes with Wnt9b expression in A/WySn. The homologous region for clf1 is on human 17q and has been implicated in human multifactorial CLP. These studies point to the WNT9B gene as a candidate and transposons as a source of the etiological complexity of human CLP.

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Cyp26a1 in embryocarcinoma differentiation: an epigenetic switch? *S. Pozzi, S. Rossetti, G. Bistulfi, N. Sacchi.*
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Retinoic acid (RA) is a signaling molecule that plays a pivotal role in vertebrate development. RA concentration is critical for the determination of the antero-posterior axis during embryonic development. RA action is mediated by specialized transcription factors, the nuclear RA receptors (RARs), that regulate the expression of genes containing a RA responsive sequence (RARE). Cyp26a1, the gene encoding the cytochrome P450 RA-specific hydroxilase, contains a RARE sequence and is critical for the development of the embryo. Here we show that Cyp26a1 is under the direct control of the RAR beta 2 receptor, which in turn is regulated by another RA receptor, RAR alpha, in pluripotent P19 embryocarcinoma cells. RNA interference of RAR beta 2 transcription in P19 cells revealed that there is a mechanistic link between RAR beta 2, Cyp26a1 regulation, and P19 differentiation, in response to RA. Thus, impairing the integration of RA-signaling through RAR beta 2 leads to stable Cyp26a1 downregulation marked by repressive epigenetic changes of Cyp26a1 chromatin. These data point at Cyp26a1 as an epigenetically regulated switch of embryocarcinoma differentiation.

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Allelic expression imbalance of *BAPX1* in patients with the oculo-auriculo-vertebral spectrum (OAVS). *S. Fischer, H.J. Lüdecke, D. Wieczorek, G. Gillessen-Kaesbach, M. Trommler, R. Kubica, B. Horsthemke.* Institut für Humangenetik, Universitätsklinikum Essen, Germany.

The oculo-auriculo-vertebral spectrum is a developmental disorder characterized by hemifacial microsomia, epibulbar tumors, ear malformations and vertebral anomalies. Most cases are sporadic, but rare familial cases suggest that OAVS has a genetic basis. To date, however, no gene defect has been identified. We have studied a patient with OAVS, multiple exostoses and a translocation 46,XX,t(4;8)(p15.3;q24.1). The translocation disrupts the multiple exostoses gene *EXT1* on chromosome 8 and the *RAB28* gene on chromosome 4. To investigate whether *RAB28* plays a role in OAVS, we sequenced this gene in 75 patients, but did not find any mutation. The *BAPX1* gene maps 76.4 kb proximal to the chromosome 4 breakpoint. As shown by studies in mice, zebrafish and Xenopus, the homeobox containing protein BAPX1 plays an important role in craniofacial development. Thus, *BAPX1* is a good positional and functional candidate gene for OAVS. We screened 105 patients for *BAPX1* mutations. In twelve patients we found six different rare nucleotide changes, three with and three without an effect on the amino acid sequence. In two families the amino acid changes were also found in non-affected relatives. We also discovered a frequent single nucleotide polymorphism in exon 1. By quantitative primer extension analysis of fibroblast RNA we found that *BAPX1* was expressed at a balanced allelic ratio in nine control individuals, but at a skewed allelic ratio in three of six patients (fishers exact test; p=0.044). In two families the unaffected father also had a skewed allelic expression ratio. Interestingly, one of these patients inherited the downregulated allele from the unaffected mother, who had a balanced allelic expression ratio, thus excluding *cis*-acting genetic variation in this case. Treatment of cultured fibroblasts from two patients with the histone deacetylase inhibitor trichostatin A, but not with the DNA methyltransferase inhibitor 5-azacytidin led to reactivation of the downregulated allele. We propose that epigenetic mechanisms causing allelic expression imbalance of *BAPX1* are involved in OAVS.

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Genome-wide Analysis of DNA Methylation in Autism Brain. *X. Zhang, T. Sahoo, Y. Jiang, A.L. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

We have proposed a mixed epigenetic and genetic and mixed de novo and inherited (MEGDI) model for autism (Am J Med Genet, 131:1, 2004). In an attempt to test this hypothesis, we have performed genome-wide analysis of DNA methylation in autism brain using the methylation-sensitive amplified fragment length polymorphism (MS-AFLP) method. DNA was digested with NotI and MseI, ligated with adapters, and amplified using PCR. This method potentially will detect two NotI-MseI PCR products from each unmethylated NotI site in the genome. The DNA was further amplified in 16 different PCR reactions with a random nucleotide added to each primer so that 1/16 of the fragments would be expected to amplify in each reaction. The NotI primer was fluorescently labeled in the final PCR, and reaction products were analyzed on the ABI gene scanner. Using this method, DNA samples from five autism brains and five control brains were compared. Perhaps surprisingly, the pattern of fragments was remarkably similar among different control brains and in comparison between control and autism brain samples. Occasional differences were identified that might represent either polymorphic restriction enzyme sites or differences in DNA methylation. Some of the changes were present in multiple autism brains but not in control brains. An additional five control brain samples and five autism samples are being analyzed, and the most promising candidate differences will be studied in greater depth. The MS-AFLP products are also being analyzed using array-CGH with human BAC DNAs. Array analysis offers the possibility for analysis of a larger number of potential methylation sites and for immediate identification of individual sites of variation or disease-related changes.

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A Whole-Genome Linkage Scan Reveals an Imprinted Locus for Wilms Tumor on Chromosome 11p15. *S. Shete[1], C. Amos[1], C. Wu[1], X. Zhou[1], L. Strong[2], V. Huff[3].* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Cancer Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX; 3) Dept Clinical Cancer Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

Wilms tumor is one of the most common solid tumors of childhood, accounting for 8% of all childhood cancers. The etiology of Wilms tumor is genetically heterogeneous. Standard linkage analyses have shown suggestive evidence of linkage on chromosome 11p15. Because this region contains clusters of imprinted genes and because approximately 70% of Wilms tumors exhibit loss of imprinting, we performed genome-wide joint linkage and imprinting analyses in nine Wilms tumor families that do not carry mutation in tumor suppressor protein WT1. One family, WTX502, had a LOD score of 3.0 for linkage at the HRAS1 locus at 11p15.5 when a software program that allows for linkage analysis in the presence of imprinting is used. The heterogeneity LOD score based on all nine families was 2.2, with the estimated proportion of linked families equal to 0.38. The difference in LOD scores using the imprinting model and standard linkage analysis was 1.4. The empirical p-value for imprinting based on these analyses was 0.0024. Our results strongly suggest the existence of an imprinted gene at chromosome 11p15.5 that is implicated in the causation of familial Wilms tumor.

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Identification of *cis*- and *trans*-acting factors modifying the risk of epimutations on chromosome 15. *C. Zogel[1], S. Böhringer[1], R. Varon[2], K. Buiting[1], B. Horsthemke[1].* 1) Institut fuer Humangenetik, Universitaetsklinikum Essen, Germany; 2) Institut fuer Humangenetik, Humboldt Universitaet Berlin, Germany.

Approximately 1-4% of patients with Prader-Willi syndrome (PWS) or Angelman syndrome (AS) have an imprinting defect on chromosome 15. In some of these patients, the incorrect imprint is the result of a microdeletion affecting the imprinting center (IC), which consists of two elements (the PWS-SRO and the AS-SRO). In most of the patients, however, the incorrect imprint is an epimutation that occurred in the absence of a DNA mutation. We have investigated whether common IC sequence variants are associated with an increased susceptibility to epimutations. We identified ten polymorphisms within the IC and found that the PWS-SRO and the AS-SRO lie on separate haplotype blocks. Using the transmission disequilibrium test, we did not observe preferential transmission of a paternal haplotype in 41 PWS trios, but found a trend for preferential maternal transmission of an AS-SRO haplotype (H-AS3) in 48 AS trios ($P = 0.058$). Furthermore, we obtained significant *P*-values for three combinations of three AS-SRO polymorphisms ($P = 0.021$), with two variants only found on H-AS3. We hypothesize that these polymorphisms affect the binding of a maternal imprinting factor and consequently the establishment of the maternal imprint. We also obtained evidence that homozygosity for the 677C>T variant of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene increases the risk of a maternal imprinting defect: the frequency of the TT genotype was significantly higher in 52 mothers of AS patients than in the patients' fathers or the general population ($P = 0.028$). MTHFR plays an important role in the synthesis of S-adenosyl-L-methionine (SAM), which serves as a methyl group donor for methyltransferases. Low SAM levels resulting from low enzymatic activity of the T variant may impair DNA methylation in the female germ line. In summary, our findings suggest that women with the IC haplotype H-AS3 or homozygosity for the *MTHFR* 677C>T variant have an increased risk of conceiving a child with an imprinting defect, although the absolute risk is low.

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Definition of the minimal regions for the imprinting phenomenon : transgenic and evolutionary approches. *A. Paoloni-Giacobino, J.R. Chaillet.* Molecular Genetics and Biochem, Pittsburgh University, Pittsburgh, PA.

Genomic imprinting is an epigenetic mechanism of gene regulation in which only one parental allele of a gene is expressed. It is a conserved phenomenon in eutherian mammals, with regards both to the genes that are imprinted and the mechanism of epigenetic inheritance leading to monoallelic gene expression. Epigenetic modifications of alleles of imprinted genes are established in oocytes and sperm, and these modifications are then inherited. Differentially methylated domains (DMDs) of imprinted genes are the genomic sites of these inherited epigenetic imprints. As such, these regions might be the imprinting backbone upon which the fundamental processes of sex-specific methylation and imprinted gene expression are built. To define the minimal DMD sequence needed for imprinting, we choose two different approaches. First, we used a well characterised RSVIgmyc transgene which has been shown to be consistently imprinted at multiple random chromosomal integration sites. By substituting the RSVIgmyc DMD with DMD sequences from endogenous mouse and human imprinted genes, we have identified the tandem repeat portions of DMDs as being required for transgene imprinting in human and mice. In the second approach, we examined the conservation of the tandem repeats during primate evolution. Surprisingly, despite the functional significance of these tandem repeats, we found that their nucleotide sequences were poorly conserved. However, their repetitiveness and high CpG dinucleotide content were found to be strikingly well conserved. Together, these observations suggest an essential role for certain CpG-rich tandem repeats in the process of genomic imprinting.

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Quantitative methylation-PCR analysis in Angelman syndrome patients with an imprinting defect. *K. Hosoki[1], K. Takano[1, 2], S. Saitoh[1].* 1) Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan; 2) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, NCNP, Kodaira, Japan.

About 2-5% of Angelman syndrome (AS) cases are caused by an imprinting defect (ID). IDs refer to a unique class of aberrant imprinting resulting in abnormal gene silencing. Most AS patients with an ID are sporadic and caused by an epimutation, while a small but significant proportion of patients carry a microdeletion in an imprinting center and inheritance is familial. Nazlican et al. (2004) reported that 30% of sporadic AS patients with an ID were associated with mosaic DNA methylation, and the ratio of normal DNA methylation was correlated with milder clinical symptoms. Thus, a quantitative methylation PCR (QmPCR) assay would be valuable for evaluating AS patients with an ID. We developed a QmPCR assay to detect the DNA methylation level at the *SNURF-SNRPN* CpG-island, a key imprinted gene in 15q11-q13. Bisulfite treatment was followed by PCR specific for the methylated and unmethylated alleles, and signal intensity was measured. We tested this method for a patient with a mosaic +idic(15)(q12), and the quantitative result was consistent with the ratio of chromosome mosaicism, confirming the usefulness of the system. We then investigated 5 sporadic AS patients with an ID, but did not detect maternal (methylated) signals in any patients. Therefore, we conclude that our series of AS patients with an ID are not associated with mosaic DNA methylation, suggesting that 30% of AS ID patients in our population have mosaic methylation. Nonetheless, the QmPCR analysis to detect DNA methylation in *SNURF-SNRPN* was reproducible and should provide a useful tool to investigate mosaicism in AS patients with an ID, and other rearrangements in 15q11-q13. Investigation of the ~ 15% AS patients with apparently normal *SNURF-SNRPN* methylation but without *UBE3A* mutations using QmPCR may uncover AS patients with a hidden ID and relatively high mosaicism, and we are now conducting such an investigation.

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Epigenetic inheritance of three distinct methylation profiles in the *H19* DMR and their correlation to monoallelic expression. *J. Tost[1], H. Jammes[2], J.-M. Dupont[2, 3], M. Fellous[2, 4], I.G. Gut[1], D. Vaiman[2].* 1) Technology Development, Centre National de Genotypage, Evry , France; 2) Unité INSERM 709, Paris, France; 3) Université Paris 5, France; 4) Université Paris 7, France.

Imprinted genes are expressed from only one of the parental chromosomes. Generally they are located in clusters and epigenetically marked by DNA methylation, histone acetylation/deacetylation and histone methylation. Their parent-of-origin specific expression is classically associated with differential methylation of specific CpG-rich DNA regions (DMRs). We characterized the methylation status of DMRs associated with *IGF2* (the DMR homologous to mouse *Igf2* DMR2) and *H19*, two imprinted genes located on human chromosome 11p15.5 in placentas and lymphocytes. Quantitative methylation analysis at each consecutive CpG dinucleotide was performed by pyrosequencing. The methylation level of successive CpGs within the *IGF2* DMR was highly variable, yielding a DMR-specific profile irrespective of the biological sample, or individual. The average methylation level was compatible with the expected hemi-methylated state. Unexpectedly, the *H19* DMR, located upstream of *H19* often departed from the expected median-methylated stage in maternal lymphocytes as well as in placental samples. Rather, three distinct methylation levels were observed that were specific for a certain individual. A predominantly monoallelic expression of *H19* was preserved independently of the DMR methylation status demonstrating that DMR allele-specific methylation and allele-specific expression of imprinted genes are not always correlated. In addition, we could demonstrate a positive correlation between the expression levels of *IGF2* and *H19* in human placenta challenging the established point of view of inverse correlation between these two genes. Mendelian transmission of the methylation profile was tested using lymphocyte DNA from related individuals. The inheritance pattern of the methylation profiles was compatible with a simple model considering one gene with three alleles constituting the first example of transmission of an effect that impacts on DNA methylation profiles in a non pathological situation with this degree of complexity.

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Beckwith-Wiedemann syndrome and Wilms tumor as a result of an epigenetic/genetic sequence rather than a single event. *D. Prawitt[1], T. Enklaar[1], C. Spangenberg[1], D. Reutzel[1], E. Lausch[1], J. Limon[2], D.E. Housman[3], J. Pelletier[4], B.U. Zabel[1].* 1) Molecular Genetics Lab, Children's Hosp/ Univ Mainz, Mainz, Germany; 2) Dept. Biology & Genetics, Medical University of Gdansk, Poland; 3) Cancer Research Center, Massachusetts Institute of Technology, Cambridge, MA, USA; 4) Dept. Biochemistry & McGill Cancer Center, McGill University, Montreal, Canada.

Genetic diseases can be caused by defects in genomic imprinting, a mechanism that normally leads to epigenetic silencing of one parental allele. A congenital overgrowth condition associated with imprinting defects in the chromosomal region 11p15.5 is Beckwith Wiedemann syndrome (BWS), with about 5% of the children developing embryonal tumors, mainly Wilms tumor (WT). Most frequently epigenetic alterations that cluster in two imprinting centers (IC), loss-of-function alterations in the *CDKN1C* gene and uniparental paternal disomy (patUPD) are observed. Hypomethylation of the proximal imprinting center (IC2), mediates silencing of the *KCNQ1* and *CDKN1C* genes. The distal IC1 is a differentially methylated repeat cluster positioned between *H19* and *IGF2*. Allelespecific binding of the zincfinger protein CTCF to IC1 regulates access of both genes to upstream enhancers. Inhibition of CTCF DNA interaction due to methylation of the CTCF binding sites on the paternal chromosome results in activation of the paternal *IGF2* promoters. Maternal microdeletions or hypermethylation of CTCF binding sites result in loss of imprinting (LOI) of *IGF2*. Consequentially biallelic *IGF2* expression has been thought to be causative for BWS. In an extraordinary familial case of BWS with WT we describe a maternally inherited IC1 microdeletion, associated with *IGF2* LOI and upregulation of *IGF2*-mRNA but without disease manifestation. As an additional event, one affected family member displays a genomic duplication of the maternal 11p15.5 allele harbouring the IC1 microdeletion. Based on our findings we propose a model in which the BWS-WT phenotype is characterized by an increase of IGF2 levels due to a defined series of (epi-)genetic lesions resulting in critical activation of the receptor signaling cascade.

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Deregulated expression of nuclear *Ube3a-ATS* upon shRNA-mediated knockdown of *Ube3a*. *M.A. Calciano, V.N. Sotirova, M. Landers, M. Lalande.* Genetics & Dev. Biol., Univ. Connecticut School of Medicine, Farmington, CT.

Failure to inherit a normal active maternal copy of the gene encoding ubiquitin protein ligase E3A (UBE3A) results in the neurogenetic disorder, Angelman syndrome. UBE3A is transcribed predominantly from the maternal allele in brain but is expressed from both alleles in most other tissues. A current hypothesis is that brain-specific silencing of the paternal UBE3A allele is mediated by a large (>500 kb) paternal noncoding antisense transcript (UBE3A-ATS). The murine P19 embryonic carcinoma (EC) cell line is a widely used model of *in vitro* neuronal differentiation and is suitable for studying certain aspects of the interaction between *Ube3a* and *Ube3a-ATS*. We have used a 7SK polymerase III promoter vector system to express shRNAs against *Ube3a* and achieved stable knockdown of *Ube3a* RNA and protein levels in P19 cells. Knockdown of *Ube3a* does not appear to affect either gross neuronal morphology during P19 cell differentiation or the expression of neuronal markers such as *Gabr3*, *-tubulin III*, *MAP2* and *Tau-1*. shRNA-mediated knockdown of *Ube3a* in P19 cells does, however, result in increased expression of *Ube3a-ATS*. The levels of the *Ube3a-ATS* transcript are augmented in the nuclear fraction of P19 cells in which *Ube3a* is knocked down, a result consistent with the predominant nuclear localization of *Ube3a-ATS* in P19 cells. The cell fractionation experiments also reveal that *Ube3a* is a predominantly nuclear transcript and that the shRNAs are efficiently silencing *Ube3a* in the nucleus. These results strongly suggest a nuclear mechanism whereby *Ube3a* modulates *Ube3a-ATS* rather than the exclusive reciprocal interaction that is common to other imprinted genes. While the nature of the sense-antisense interaction at the *Ube3a* locus remains to be elucidated, we favor a model whereby *Ube3a* represses *Ube3a-ATS* via a transcriptional or chromatin silencing mechanism. We also report that up-regulation of *Ube3a-ATS* is observed in a mouse model of Angelman syndrome and suggest that the increased levels of *Ube3a-ATS* may contribute to the phenotypic manifestations of this neurogenetic disorder.

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Genomic imprinting of *Ube3a* is not entirely dependent on an antisense RNA. *D.C. Colosi, V.N. Sotirova, M. Lalande.* Dept. of Genetics and Developmental Biology, University of Connecticut, Farmington, CT.

The majority of Angelman syndrome (AS) cases result from the failure to inherit a normal maternal allele of the imprinted gene encoding ubiquitin ligase E3A (UBE3A). In normal brain (mainly hippocampus, cerebellum, olfactory bulb), *Ube3a* is expressed predominantly from the maternal allele, while a non-coding antisense RNA (*Ube3a-ATS*) is exclusively transcribed from the paternal allele. The current hypothesis is that the brain-specific *Ube3a-ATS* silences the paternal *Ube3a* in cis.

We are studying the imprinted expression of *Ube3a* in R1 murine embryonic stem (ES) cells. The R1 ES cell line is derived from a cross between the 129/SvJ and 129/Sv-+$^{Tyr+p}$ mouse strains. In the paternal 129/Sv-+$^{Tyr+p}$ strain, a segment of chromosome 7 that includes all genes from the AS/Prader-Willi syndrome critical region, is of non-129 origin. As a result, the parental *Ube3a* alleles can be discriminated using single nucleotide polymorphisms. Upon retinoic acid-stimulated neuronal differentiation of R1 ES cells, *Ube3a-ATS* transcription is detectable by seven days after induction.

Here we report that, in undifferentiated R1 ES cells and at early time points after induction, expression of *Ube3a* is predominantly from the maternal allele. Since *Ube3a-ATS* is not expressed until later during the process of neuronal differentiation, our data strongly suggest that imprinting of Ube3a does not strictly depend on the expression of *Ube3a-ATS*. However, a more marked repression of *Ube3a*, and a decreased paternal contribution is observed in the ES cell derived-neurons that co-express *Ube3a-ATS*. Our studies suggest that the mechanism of imprinting of *Ube3a* in stem cells, and during the very early stages of differentiation is independent of *Ube3a-ATS*. in fully differentiated neurons, however, it appears that *Ube3a-ATS* plays a role in enhancing repression of the paternal *Ube3a* allele. These findings suggest the possibility of distinct imprinting mechanisms for *Ube3a* in stem cells and stem cell-derived neuronal cells. Supported by NIH R01-NSO30628 and T32DE07302.

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Genome-wide methylation in MZ twin pairs concordant and discordant for reading ability using an optimized oligonucleotide array. *E. Hatchwell[1], Y. Wang[1], T. Richmond[2], R. Plomin[3], I.W. Craig[3].* 1) Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA; 2) NimbleGen Systems Inc., One Science Court, Madison, WI 53711, USA; 3) SGDP Centre, Box PO 82, Institute of Psychiatry, Denmark Hill, London SE5, UK.

Discordance in monozygotic (MZ) twins is a widely observed phenomenon but one for which few explanations are generally lacking. We have studied genome-wide methylation patterns in 8 MZ twin pairs, 4 concordant and 4 discordant for reading ability. Our pilot study has two broad aims: 1. To estimate the level of DNA methylation variability in MZ twins in general; 2. To search for genomic regions whose methylation pattern is dramatically, and abnormally altered in our discordant twin pairs. We have designed and optimized a genome wide oligonucleotide array, which interrogates MspI/HpaII sites distributed throughout the genome. Our basic design approach is as follows: 1. In silico restriction analysis was used to generate a list of small (200-800bp) MspI fragments; 2. Arrays containing 50-mer oligonucleotides recognizing these small MspI fragments, and chosen to be as non-repetitive as possible, were synthesized using Maskless Array Synthesis (MAS) technology (NimbleGen Systems Inc, Madison); 3. A subset of all 50-mers was selected for the final design on the basis of ratiometric changes observed after pre-digestion of HpaII fragments with a variety of frequent 4-cutters. For this approach to be successful, it was necessary to optimize the method of generating HpaII representations from small amounts of starting material. This was achieved in a 2-step PCR protocol. Briefly, genomic DNA was digested with HpaII, adapters ligated and material amplified by PCR in two stages. Our protocol results in the amplification of fragments in the range 200-800bp, closely mimicking the design of the arrays. Our preliminary evidence suggests the presence of wide variability in methylation status at many genomic sites. We are currently validating a representative portion of altered HpaII sites using pyrosequencing and other methodologies.

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Quantitative Methyl Sensitive PCR as an Alternative to Southern Analysis for Molecular Diagnosis of Beckwith-Wiedemann Syndrome. *B. Coffee, K. Muralidharan.* Dept Human Genetics, Emory University, Atlanta, GA.

Beckwith-Wiedemann Syndrome (BWS) and isolated hemihyperplasia are associated with DNA methylation defects at 11p15. The DNA methylation defects can occur at one of two differentially methylated regions (DMRs) located immediately upstream of the H19 gene or in the promoter of the Lit1 gene (referred to as DMR1 and DMR2, respectively). About 50% of the cases of BWS have loss of DNA methylation at the Lit1 promoter, whereas only 2% of the cases have identifiable hypermethylation at the H19 locus. Another 10-20% of cases are due to paternal uniparental disomy (UPD) of 11p15. Currently, molecular diagnosis of BWS testing is done by assessment of DNA methylation by Southern blot analysis using methylation sensitive restriction enzymes. Here we describe the use of a quantitative methyl sensitive PCR (MSP) as an alternative to Southern analysis. This method takes advantage of the differential deamination of cytosine and 5-methylcytosine to uracil by sodium bisulfite allowing the distinction between methylated and unmethylated DNA. This method will detect both loss of Lit1 DNA methylation as well as paternal 11p15 UPD, accounting for 60-70% of the cases of BWS and the majority of cases of isolated hemihyperplasia. The advantages of the quantitative MSP method is that it requires far less DNA, is less labor intensive and has a faster turn around time than the traditional Southern method.

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Analysis of Epigenetic Control of *MECP2* in Autism. *M. Shinawi[1], A. Haldipur[1], YH. Jiang[1], R. Stevenson[2], AL. Beaudet[1].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Greenwood Genetic Center, South Carolina 29646, USA.

Genetic predisposition to autism is evident from family and twin studies. So far, genome-wide linkage studies have failed to provide a strong evidence for major autism-related loci. Male sex is the strongest and most consistent risk factor in autism. *MECP2* is an X-linked gene encoding a methyl-CpG binding protein that has been implicated recently in regulating genes involved in social behavior. Furthermore, there is evidence that duplication of *MECP2* (the Rett gene) with presumptive over-expression causes neurologic abnormalities in males. Despite the resemblance between the phenotypes of autism and Rett, *MECP2* mutations are an infrequent cause of essential autism. We are testing the hypothesis that epigenetic abnormalities (epimutations) rather than mutations of *MECP2* might be a more common cause of autism. We analyzed the methylation of the CpG island at the promoter of *MECP2* in lymphoblast cell lines of 44 autistic males and 14 autistic females from the AGRE, NIMH, and South Carolina Autism Project collections, and compared these to 28 controls (13 females and 15 males). In addition, brain samples from 8 autistic individuals were examined and compared to 5 normal brains. The methylation status was tested by Southern blot analysis after digestion with methylation sensitive enzyme. In all lymphoblast and brain samples from males, the DNA was completely unmethylated. For all brain samples and lymphoblast lines from control females and for most lymphoblast lines from females with autism, the data were consistent with the interpretation that the inactive X chromosome was methylated and the active X unmethylated. However, two lymphoblast cell lines from unrelated females with autism demonstrated hypomethylation. Additional studies are underway to test more brain samples, to perform ChIP assay, to precisely evaluate the DNA methylation by bisulfite sequencing, and to test fresh blood samples to avoid the possible artifacts of methylation arising in cell culture. We propose that epigenetic dysregulation of *MECP2* could be a causative factor in some fraction of autistic patients.

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Comprehensive DNA Methylation Profiling by Real-time Quantitative PCR. *M.O. Dorschner, M. Weaver, A. Schafer, K. Lee, J. Neri, J. Goldy, J.A. Stamatoyannopoulos.* Molecular Genetics, Regulome Corporation, Seattle, WA.

We have developed a high-throughput methylation profiling technology based on real-time quantitative PCR (HMqPCR) and the sensitivity of the restriction enzyme, McrBC to DNA methylation. Large genomic segments can be rapidly scanned for methylation sensitivity at 250 bp intervals by interrogating a region of interest with a series of contiguous qPCR assays. Methylation sensitivity is expressed quantitatively as a copy number ratio of the McrBC-digested versus undigested DNA and reported as a methylation score ranging from 0 to 1. After plotting methylation scores according to genomic position, a sophisticated outlier detection algorithm is employed to identify regions of methylation sensitivity. To demonstrate the capability of our assay to detect localized hypo- and hypermethylation, we applied this technique to two 500kb regions of the human genome, one containing the beta globin (HBB) locus and the other, insulin growth factor 2 (IGF2). A 15kb region harboring the ^{A} and ^{G} genes, was found completely unmethylated in cell line K562, while the beta- and delta globin promoters were heavily methylated. A large region of hypermethylation was observed immediately upstream of the beta globin LCR. This region coincides with a chromatin domain boundary. A differentially methylated region (DMR) of ~25kb, located downstream of IGF2 was found completely unmethylated in transformed lymphoblasts. This segment corresponds precisely to a DMR known to play a role in imprinting. The regions flanking the DMR contained many sites of localized hyper- and hypomethylation, as well as a number of methylation hotspots. Methylation of repetitive DNAs was remarkably heterogeneous. HMqPCR can be used: 1) to identify DMRs, 2) to localize sites exhibiting inter-individual variation in methylation, 3) to identify tissue-specific sites of methylation, 4) to establish detailed methylation profiles of large genomic regions and 5) to develop biomarkers for the molecular diagnosis of disease. All these goals are critical to our understanding of epigenetic control of gene expression and how aberrant DNA methylation contributes to human genetic disease.

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High-throughput detection and quantification of CpG sites methylation levels in the genome using MASSCleave technology. *I. Ragoussis[1], L. Marcelline[1], V. Viprakasit[2, 3], S. Smiley[2], R. Gibbons[2], D. Higgs[2], S. Collela[1].* 1) Wellcome Trust Ctr Human Gen, Univ Oxford, Oxford, United Kingdom; 2) 2MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK; 3) 3Department of Pediatrics and Siriraj-Thalassemia Research Program, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Epigenetics contribution to human disease is very well recognized, but not completely understood. Epigenomics, i.e. the whole-genome study of epigenetic variation, is possible thanks to new technologies and the availability of genome sequences. We used MassCLEAVE technology (Sequenom) to detect methylation at multiple CpG sites in a given DNA sequence. This protocol, originally developed for SNP discovery, can be used to detect the C to T chemically induced modification after bisulfite treatment at CpG sites in the genome sequence (where a C will be detected if methylation is present). We focused our analysis on a number of CpG sites known to be affected by methylation; including the INK4A (p16) promoter and three sites in the -globin cluster on chromosome 16. The MASSCleave protocol on bisulfite treated DNA readily detects the methylation status of multiple CpG sites interrogated in a single amplicon. We used serial dilution experiments to test the sensitivity of the method in the quantification of methylation levels. To evaluate the quantification potential of MASSCleave approach, we compared our data with the established Allelotyping protocol for some of the CpG sites for which we could design MASSExtend assays. We performed statistical analyses to compare the performance of these two approaches in DNA methylation profiling. In conclusion, MassCLEAVE offers a valuable platform for high-throughput methylation analysis and allows semi-quantitative analysis of the methylation levels. The implementation of robust high-throughput epigenomics profiling methods is paramount for a better understanding of the role of epigenetics alteration to complex diseases susceptibility.

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Direct comparison of two different strategies to sequence bisulfite-treated genomic DNA for DNA methylation analysis in genetic diseases and cancer samples. *M.F. Fraga[1], A. Rico[2], B. Finkelnburg[3], M. Esteller[1].* 1) Epigenetics Laboratory, Spanish National Cancer Center, Madrid, Madrid, Spain; 2) Applera France, Applied Biosystems; 3) Applera Deutschland GmbH, Applied Biosystems.

Enzymatic methylation on the cytosine of genomic DNA (gDNA), specifically methylation of cytosines (C) at CpG motifs located in the CpG island promoter regions, shuts down gene expression. A well known method to study methylation patterns is to treat gDNA by sodium bisulfite to distinguish methylated cytosine (5mC) from unmethylated C, which is deaminated to uracil (U) and replaced by thymine (T) in subsequent amplification. 5mC still remains as C. Subsequent amplification can focus on selective amplification of methylation patterns in CpG islands (methyation specific PCR, MSP) or on amplification of bisulfite treated (converted) gDNA (Bisulfite treatment specific PCR, BIS). Selection of PCR focus is done by primer design. After PCR, sequencing can clarify the methylation pattern. Due to very imbalanced nucleotide composition after bisulfite treatment bias in amplification of bisulfite treated gDNA as well as in sequencing products may lead to experimental problems. PCR improvements during amplification of bisulfite-converted gDNA by the use of universal tailed primers, combined with changes in thermal cycling and reactant concentrations are described earlier. These PCR products can be sequenced by using the universal primer tails. This method will be compared to the cloning of BIS PCR products for sequencing the bacterial clones. For the whole comparison two different primer design softwares were used to generate primer sets for 2 regions of the BRCA1 gene. All primer sets will be used untailed as well as with universal tails for direct sequencing of PCR products. Only untailed PCR products will be cloned. The rate of success will measured by number of steps necessary to generate a specific PCR product, number of steps to generate specific sequences and quality of sequences. Finally we will discuss the usefulness of both methods with respect to specific issues targeted by the scientific community, e.g. quantification of methylation.

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Promoter methylation of the TSLC1/IGSF4 and DAL-1/4.1B genes involving a novel tumor suppressor cascade in human non-small cell lung cancer detected by bi-sulfite SSCP analysis. *Y. Murakami[1], S. Kikuchi[1], D. Yamada[1], T. Fukami[1], M. Masuda[1], M. Sakurai-Yageta[1], Y.N. Williams[1], T. Maruyama[1], H. Asamura[2], Y. Matsuno[3].* 1) Tumor Suppression Project, Natl Cancer Ctr Research Inst, Tokyo, Japan; 2) Dept. Thoracic Surgery, Natl. Cancer Ctr Hospital, Tokyo, Japan; 3) Dept. Diagniostic Pathology, Natl. Cancer Ctr Hospital, Tokyo, Japan.

TSLC1/IGSF4 is a tumor suppressor gene in human non-small cell lung cancer (NSCLC) that we have previously identified by functional complementation. TSLC1/IGSF4 encodes an immunoglobulin-like cell adhesion molecule and is expressed in most epithelial tissues including the lung, while the expression is lost in many human cancers including NSCLC. We also identified that TSLC1/IGSF4 protein associated with an actin-binding protein, DAL-1/protein 4.1B, through its cytoplasmic domain, suggesting that TSLC1/IGSF4 and DAL-1/4.1B cascade is involved in cell adhesion coupled with cytoskeleton organization. In the present study, we examined the methylation status of the TSLC1/IGSF4 and DAL-1/4.1B genes by bisulfite-SSCP analysis. Bisulfite-SSCP is a sensitive method for the quantitative detection of the methylation status on the basis of the allelic information. Using this method, we found that the promoters of the TSLC1/IGSF4 and DAL-1/4.1B genes were methylated in 46 (45%) and 59 (57%) of 103 primary NSCLC, respectively. Methylations of the TSLC1/IGSF4 and DAL-1/4.1B promoters were significantly associated with a shorter disease-free survival in adenocarcinoma patients (p=0.049 and p=0.0011, respectively). Furthermore, DAL-1 methylation was observed preferentially in tumors with advanced stages in adenocarcinomas (p=0.0026). On the other hand, the TSLC1/IGSF4 methylation was significantly associated with heavy smoking (Brinkman index >800) (P=0.0054). Finally, about 70% of primary NSCLC tumors represented epigenetic inactivation of either the TSLC1/IGSF4 or the DAL-1/4.1B gene. These results strongly suggest that the aberration of the TSLC1/IGSF4 and DAL-1/4.1B cascade is deeply involved in the majority of primary NSCLC tumors and provides an indicator for poor prognosis.

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High-throughput DNA methylation profiling using universal bead arrays. *M. Bibikova[1], Z. Lin[2], L. Zhou[1], E. Chudin[1], E. Wickham[1], B. Wu[1], N. Thomas[2], Y. Wang[2], C. Seifart[3], D. Barker[1], M. Chee[1], J. Floros[2], J. Fan[1].* 1) Illumina Inc., San Diego, CA; 2) Penn State University, Hershey, PA; 3) Philipps-University of Marburg, Germany.

We developed a multiplexed DNA methylation detection method based on high-throughput genotyping of bisulfite-converted genomic DNA. The technology uses Illuminas standard GoldenGate assay format and BeadArray^{TM} technology platform for genetic analysis. We developed a set of internal controls to monitor the assay performance, and demonstrated that current assay sensitivity and specificity is sufficient to detect changes in methylation status at more than 1,000 different sites simultaneously with 200 nanograms of human genomic DNA. Reproducible DNA methylation profiles were obtained within replicates (an average R^2 of 0.98). We estimated that our assay can resolve a 10% methylation difference between samples with 95% confidence.

We used the assay developed for 370 genes for methylation profiling in seven normal DNA samples derived from different tissues and 17 colon, breast, lung and prostate cancer cell lines. All cancer samples were correctly separated from normal, and highly specific methylation signatures were obtained for each cancer type. Methylation levels of five loci in six lung cancer cell lines and normal lung tissue were validated using methylation-specific PCR.

We applied this technology to DNA methylation analyses in lung cancer. We analyzed the methylation status of 370 genes (>1500 CpG sites) in 46 lung cancer and normal tissue samples, to discover methylation-based biomarkers for lung cancer. Panels of squamous cell carcinoma specific and adenocarcinoma specific methylation markers were identified at high confidence ($P < 0.001$). The markers identified for the adenocarcinoma were further validated in an independent sample set ($N = 24$). This technology should prove useful for DNA methylation analyses in large populations, with potential application to the classification and diagnosis of a broad range of cancers.

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Self-association of human DNMT3A and DNMT3B DNA methyltransferases. *Z.X. Chen, A.D. Riggs.* Division of Biology, Beckman Research Institute of the City of Hope, Duarte, California, 91010.

Proper control of DNA methylation patterns is essential for mammalian development. Abnormal methylation patterns have been linked to cancer and several human genetic diseases. Two de novo DNA methyltransferases, Dnmt3a and Dnmt3b, have been shown to be required for the establishment of DNA methylation patterns in germ cells and early embryos. The mechanism by which DNMT3A and DNMT3B carry out de novo methylation remains largely unknown. Here, we demonstrate, using the yeast two-hybrid system and co-immunoprecipitation analysis, that human DNMT3A and DNMT3B are capable of self-association. Deletion analysis revealed that the C-terminal catalytic domain is responsible for the self-interaction. Since most mutations causing immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome occur in the catalytic domain of DNMT3B, we investigated whether these mutations can affect the ability of DNMT3B to self-interact. Two DNMT3B ICF mutants (H814R and D817G) show reduced ability to interact with themselves in the yeast two-hybrid system. In addition, DNMT3A can interact with DNMT3B through their C-terminal catalytic domains. Taken together, these results suggest that both DNMT3A and DNMT3B might functionally exist as dimers or higher-order oligomers. We propose that the self-association of catalytic domain may play a role in regulating activity and function of DNMT3 methyltransferases.

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Epigenetic profiling of CpG islands in sporadic breast cancers : a pilot study. *J. Flanagan[1], N. Waddel[2], S.C. Wang[1], P. Simpson[3], S.R. Lakhani[3], A. Petronis[1], G. Chenevix-Trench[2].* 1) Dept Neurogenetics, Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) Queensland Institute of Medical Research, Herston, Brisbane, Australia; 3) Department of Pathology, University of QLD. Brisbane, Australia.

There is a large body evidence for an involvement of methylation of an increasing number of genes in sporadic breast cancer. This pilot study aimed to assess microarray based epigenetic profiling for the identification of differentially methylated genes in sporadic breast cancer. Hypomethylated DNA was enriched using methylation sensitive restriction digestion and adaptor mediated PCR from pairs of macrodissected tumor cells and normal breast epithelium (N=5). These enriched fractions were hybridised in duplicate to CpG Island microarrays containing 12,192 individual CpG island clones from the Sanger Institute. A modified CGH protocol was also performed to discriminate DNA sequence variations (SNPs, deletions, insertions) from methylation variations. Validation of the arrays was performed using bisulphite modifification and primer extension using the ABI SNaPshot assay. There was considerable heterogeneity among the breast tumour/normal pairs with 65-203 out of 12,000 CGIs identified as methylated in each tumour relative to the matching normal breast. However, combined data for the 5 tumours show 13 CpG islands methylated in normal but not tumour, and 39 CpG islands methylated in tumour but not normal. The CGH analysis identified 845 spots that show genomic gain in the tumour samples compared to the normal and 63 spots that show genomic loss in the tumour sample compared to normal. Combining the methylation and CGH data identified 10 CpG islands methylated in normal but not tumour and 15 CpG islands methylated in tumour but not normal. The SNaPshot assay validated 8/13 spots suggesting a validation of approximately 60%. This pilot study has shown that microarray-based epigenetic profiling can identify differentially methylated genes in breast tumor DNA. This study also highlights the importance of discriminating between SNPs and real methylation differences when using methylation sensitive enzymes.

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Deconstructing position effects: *SOX9* regulation via the Shh pathway and distal regulatory elements. *G.A. Bien-Willner[1], P. Stankiewicz[1], J.R. Lupski[1,2,3].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Position effect, or the change in a genes expression resulting in patient phenotype without intragenic mutations, has been described in patients with chromosome translocations even when the breakpoints occur at some distance from the causative gene. This has resulted in speculation of the importance of chromatin interactions occurring upwards of a megabase proximal or distal from the gene, and effecting its expression. *SOX9* is one such gene susceptible to position effects; it is responsible for male sexual determination and bone formation. Several patients with campomelic dysplasia (CD) have been described without *SOX9* mutations and with translocation breakpoints as far as 932 kb from the gene. Studying one such case (900 kb upstream *SOX9*) led to the prediction of two possible *cis*-acting regulatory elements of *SOX9*, named SOX9cre1 and SOX9cre2, located 1.1 Mb proximal to *SOX9*. Here, we investigate the possible role of these regulators in *SOX9* expression using reporter assays in the *SOX9*-expressing chondrosarcoma cell line SW1353, and assess their role in the tissue-specific activation of the gene. SOX9cre1 and SOX9cre2 increase the expressional activity of a minimal *SOX9* promoter nearly two-fold, consistent with an enhancer role. *In silico* studies identify a GLI1 binding site in the 2.1 Kb SOX9cre1, suggesting that *SOX9* may be activated directly through the sonic hedgehog (Shh) signaling pathway. The implication of the Shh signaling pathway in *SOX9* expression prompts us to test these regulators potential interactions with Gli1, Nkx3.2, and BMP in EMSA studies, as well as locate other long-range regulators of *SOX9* utilizing ChIP-on-chip experiments. Our experiments are further revealing the sea of regulation that lies in the gene desert proximal to *SOX9*.

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Deletion of the endogenous *HPRT* promoter on the active X chromosome leads to complex alterations of histone modification patterns. *S. Rodriguez-Jato, J. Shan, S.L. Kang, J.O. Brant, A. Heggestad, T.P. Yang.* Dept. Biochemistry and Molecular Biology, College of Medicine Univ. Florida, Gainesville, FL. 32610.

Transcriptionally active and repressed genes are associated with distinct patterns of histone modifications. Promoter regions and their associated factors have been postulated to recruit modifying enzymes that establish and/or maintain these patterns. We are examining the role of the promoter in maintaining transcriptionally active epigenetic states. We targeted a 323 bp deletion of the endogenous *HPRT* promoter on the active X chromosome in human HT1080 cells which removed nearly all known transcription factor binding sites in the promoter and abolished transcription. Previous analysis of this deletion showed loss of the DNase I hypersensitive site in the *HPRT* promoter region, but little or no change in general DNase I sensitivity or DNA methylation patterns across the locus. We now have examined the effect of this deletion on histone modification patterns using ChIP assays on the control parental and mutated cells. Analysis of nine sites across the wild type locus detected high levels of dimethylated H3 lysine 4 (H3-K4diMe) over the promoter and 5 transcribed region which decreased to background levels ~12 kb downstream of the promoter. Additionally, high levels of acetylated H4 (H4 Ac), H3-K9Ac and H3-K4triMe in control wild type cells were confined to the 5 transcribed region. Deletion of the minimal promoter resulted in a significant decrease in the levels of H3-K9Ac and H3-K4triMe. However, the mutation also led to an increase in H4Ac levels (predominantly in the 5 flanking region), and had no effect on H3-K4diMe levels. Our data suggest that H4 hyperacetylation and H3-K4diMe are not dependent on regulatory elements in the minimal promoter (and/or active transcription), and are not sufficient to maintain DNase I hypersensitivity of chromatin in the region. In contrast, maintaining H3-K9Ac and H3-K4triMe appear to require one or more regulatory elements associated with the minimal promoter region and/or active transcription. Thus, different mechanisms may be involved in maintaining specific types of histone modification patterns on active genes.

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RNAi is an intrinsic mechanism for human L1 retrotransposon control. *N. Yang, H.H. Kazazian.* Dept Genetics, Univ Pennsylvania, Philadelphia, PA.

LINE-1s or L1s are highly abundant retrotransposons comprising ~17% of the human genome. A full-length human L1 (L1Hs) is ~6 kb, containing a 5UTR, two ORFs and a 3UTR. As an autonomous non-LTR retrotransposon, L1 moves by a copy and paste mechanism, i.e., the precursor L1 is transcribed into RNA and then reverse transcribed and integrated into a different locus of the genome. Most L1Hs are retrotransposition-defective due to 5 truncation, inversion or mutation, still there are ~5,000 full-length L1Hs and ~100 of them are potentially retrotransposition-competent in the average diploid genome. Although driving genome evolution in constructive ways, L1 retrotransposition can also be detrimental to the host and has to be controlled. Since transcription of L1Hs starts around the +1nt of 5UTR and an antisense promoter (ASP) has been reported to be located within the 5UTR, we tested the hypothesis that bi-directional transcripts of L1Hs 5UTR could produce small interfering RNAs (siRNAs) and reduce retrotransposition via an RNAi mechanism. In the present work, we successfully detected the antisense transcript driven by ASP, mapped its start site by 5RACE and found L1Hs specific ~21nt siRNAs using Northern blot. To facilitate further study, we used a Flp-In system and established stable cell lines expressing the d2EGFP gene driven by a variety of 5UTR fragments. We found that deletion of the ASP promoter region increased d2EGFP expression in established cell lines and increased L1Hs retrotransposition activity in a cell culture based assay. Finally, targeting of Dicer1, a key component in RNAi pathway, almost doubled the retrotransposition activity of full-length L1Hs; however, this effect was abolished by the deletion of the ASP promoter region. In conclusion, we provide the first evidence that an RNAi mechanism triggered by antisense transcript may control retrotransposition of full-length L1Hs in an efficient and economic way. Therefore, RNAi may be an evolutionarily conserved mechanism of genome defense against invading nucleic acids, such as transposons and viruses, even in mammalian systems. Our work may also shed light on the understanding of the regulatory role of abundant antisense transcripts in the eukaryotic genome.

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Examining the question of chromosome counting in X inactivation using human triploid fibroblasts. *S.M. Gartler[1,2], K.R. Varadarajan[2], P. Luo[2], T.K. Canfield[2], D.K. Barden[3], R.S. Hansen[2].* 1) Dept. Genome Sciences Univ. Washington, Seattle, WA; 2) Div. Medical Genetics, Univ. Washington, Seattle, WA; 3) Dept Laboratory Medicine, Univ. Washington, Seattle, WA.

Dosage compensation in mammals is achieved by the transcriptional silencing of one of the two X chromosomes in the female early in development (X chromosome inactivation; XCI). It is widely assumed that an early event in XCI is a counting step that results in one active X chromosome per diploid autosomal set, with any additional Xs rendered inactive. For normal female diploids and X chromosome aneuploids, however, there is no need to assume a chromosome counting step to achieve this ratio. Simply marking one X for active status would be sufficient if inactivity is the default state for all remaining Xs. To critically evaluate the chromosome counting idea, a system is needed in which the one active X per diploid autosomal set cannot be achieved, such as in triploids. If chromosome counting is involved, XCI in triploids is expected to be unstable, as suggested by Jacobs et al. (Am J Hum Genet 31:446-457, 1979), particularly during the developmental window when XCI is established. Several human triploid cultures are mosaic with respect to the number of inactive X chromosomes. This mosaicism could result from: 1) XCI instability because of the abnormal X:autosome counting problem, 2) stochastic XCI because of a limiting autosomal factor involved in a threshold mechanism, or 3) a one-X-active marking mechanism followed by inactive X chromosome loss and cell selection. Our data on cultures of mosaic triploid fibroblasts and derived clones excludes the one-X-active model, implying that the XCI mosaicism seen in triploids reflects choices made during X inactivation. These data, therefore, are compatible with XCI models involving X:autosome counting and/or threshold mechansims for marking the active X using autosomal factors.

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Skewed XCI leads to the expression of Hemophilia A in three heterozygous females in an Atlantic Canadian kindred. *N. Renault[1], S. Dyack[2], M. Dobson[3], T. Costa[4], W. Greer[1].* 1) Pathology; 2) Medicine; 3) Biochemistry, Dalhousie University, Halifax, NS; 4) Medical Genetics, Ste-Justine Hospital, Montreal, QE.

Background: A family presented with 3 heterozygous females affected with hemophilia A (HA), an X-linked recessive (SXR) bleeding disorder typically expressed in males and homozygous females. In rare cases, skewed X-chromosome inactivation (XCI) in heterozygous women has led to the expression of HA and other SXR disorders. Our objective is to elucidate the mechanism of HA expression in these heterozygous females.

Hypothesis: We hypothesize that our participants (ppt) are affected with HA because of skewed XCI, and, since multiple females in the family are affected, the skewing is due to genetics, rather than to random chance.

Results: We assessed 14 ppt for the factor VIII gene (F8) inversion common in severe HA. We found 2 males, and 6 carrier females with the type II inversion, and 6 normals. We measured factor VIII levels in 14 ppt and found 3 with severe HA (2 males, 1 female), and 2 females with mild HA. 9 females were informative for XCI skewing at the HUMARA locus. 4 had severe skewing (>80%) in peripheral blood lymphocytes. The factor VIII levels in carrier females correspond to the prevalence of active Xs containing normal F8 in all cases. Preliminary evaluation of buccal mucosa suggests that skewing at this site does not differ significantly from blood. Through microsatellite analyses, we have identified a region, Xq24-q27, that segregates with the skewing phenotype in this family. This contains a region (Xq24-25) previously associated with familial XCI skewing.

Conclusions: Skewed XCI is the mechanism by which 3 heterozygous females are expressing HA, furthermore, in accordance with the literature, we feel that since there are 4 females skewed >80%, the skewing observed is due to genetic control.**Future directions:** More ppt have been recruited and more microsatellites are being analyzed to narrow down the critical region, and we are exploring the development of a cell culture system for analyzing XCI skewing.**Funding:Killam Foundation, CIHR, CDHA**.

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Genetic Study of Glaucoma and Age-Related Macular Degeneration Using the Utah Population Database. *J. Baird[1,2], N. Zabriskie[2], P.S. Bernstein[2], Z. Yang[1], K. Zhang[1,2], Y. Yao[1], D. Gibbs[1], J. Meyer[1], R. Gomez[1].* 1) Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Ophthalmology and Visual Sciences, University of Utah, Salt Lake City, UT.

Introduction: Age-related macular degeneration (AMD) and primary open-angle glaucoma (POAG) are two leading causes of blindness in the US and the developed world. Genetic predisposition plays an important role in AMD and POAG, but identification of genes for these two conditions has been hampered by the multi-factorial nature of the disease. The Utah Population Database (UPDB) was created by the University of Utah and contains over 20 million records. It is made up of many data sets including genealogies, birth and death certificates, and drivers license data. There are two major data sets from which family members can be identified: 1.6 million genealogy records and 1.8 million Utah birth certificates. The genealogy records can span as many as eleven generations. **Methods:** We searched the medical record database of the University of Utah Hospitals and Clinics and identified patients with AMD and glaucoma. We then performed cross-match analysis with genealogy records at the UPDB to identify and recruit extended families with clustering of AMD and glaucoma. **Results:** We identified 5,172 AMD and 4,328 POAG patients from 1.5 million medical records. Cross-matching and clustering analyses with the UPDB identified 15 extended large families with AMD and 14 with POAG using our criteria of having five or more living affected members within each family. We have also established B lymphoblastoid cell lined for 454 AMD patients, 54 POAG patients, and 270 normal age-matched controls. **Discussion:** We have identified large pedigrees with multiple affected members with AMD and POAG. The resources established will aid greatly in our effort to identify genes causing these diseases.

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Inversion at the X-linked FLN-EMD locus is common and recurrent. *D.L. Nelson[1], N. Lamb[2], S.T. Warren[2], P.E. Bonnen[1,3].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Human Genetics, Emory University School of Medicine, Atlanta, GA; 3) Rockefeller University, New York, NY.

Genomic rearrangements are increasingly recognized for their role in susceptibility to disease and in genetic variation. The question of whether such rearrangements are stable enough to be transmitted and tracked in studies for disease susceptibility remains unresolved, and there is little data regarding the frequency of inversions in humans. The first polymorphic inversion described in humans comprises a 48 kb region spanning the emerin (EMD) and filamin (FLN1) genes located in Xq28. This region is flanked by 11.3 kb inverted repeats that share greater than 99% sequence identity. The two alternative orientations of this region were found at an ~80:20 ratio in X chromosomes from a largely Caucasian population by Southern blot studies (Small et al. 1997). We have extended these data to characterize the stability of the EMD/FLN1 inversion by genotyping worldwide populations for the inversion and for six SNPs that span the inversion region. We characterized 430 individuals (681 X chromosomes) from 41 different populations for the EMD/FLN1 inversion. The majority of individuals typed have the cen-FLN1/EMD-ter orientation. However, nearly all Africans typed have the cen-EMD/FLN1-ter orientation making this the most likely ancestral orientation. Genotypes were obtained for 268 males (of the 430 total individuals) who were typed for the EMD/FLN1 inversion for six SNPs spanning 131 kb surrounding the inversion. Haplotype analysis shows both orientations of the inversion to be present on multiple haplotypic backgrounds indicating recurrence or instability of this inversion. Genotyping the inversion in 10 human sperm DNA samples showed the presence of both orientations in all individuals typed, despite the presence of a single orientation in DNA derived from blood samples in the same individuals. Thus, both population genetic and direct analysis of germ cells indicate suggest that the EMD/FLN1 inversion polymorphism is recurrent.

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Extensive traffic of genetic information between segmental duplications flanking the typical 22q11.2 deletion in velo-cardio-facial syndrome/DiGeorge syndrome. *A. Pavlicek[1], R. House[2], A.J. Gentles[1], J. Jurka[1], B.E. Morrow[3].* 1) Genetic Information Research Institute 1925 Landings Drive Mountain View, CA 94043; 2) Department of Biochemistry Albert Einstein College of Medicine 1300 Morris Park Avenue Bronx, New York, 10461; 3) Department of Molecular Genetics Albert Einstein College of Medicine 1300 Morris Park Avenue Bronx, New York, 10461.

Segmental duplications or low copy repeats (LCRs) are highly unstable segments in the human genome prone to frequent recombinations. To delineate regions in LCRs that might contain hotspots for genomic rearrangements, we generated an algorithm to detect sites of altered recombination by searching for polymorphic positions in BAC clones mapped to the LCRs. This method distinguishes single nucleotide polymorphisms from paralogous sequence variants and complex polymorphic positions. Sites of shared polymorphism are considered potential sites of gene conversion or double cross-over between the LCRs. We applied this method to two 240 kb long low copy repeats termed LCR22-2 and LCR22-4, which flank the typical 22q11.2 deletion in velo-cardio-facial syndrome/DiGeorge syndrome. After excluding gaps and highly unstable simple repeats, alignment of well-mapped BAC clones spanning these regions revealed 2,308 polymorphic positions. We found an inverse correlation between regions of paralogous sequence variants that are unique to a given position within one LCR22 and clusters of shared polymorphic sites. This, together with the presence of both LCRs in the chimpanzee genome suggest that these clusters are products of past transfers of DNA information between the LCR22s and not remnants of ancestral single nucleotide polymorphisms. Clusters of shared polymorphic sites depleted in paralogous sequence variants predict regions especially prone to genomic rearrangements in the two LCR22s. Interestingly, we found that recombination/gene conversion can transfer indels up to 1.5 kb long between LCR22s. Nucleotide identity seems be the major factor discriminating recombining from non-recombining regions. Finally, we propose that high levels of genetic exchanges may be hallmarks of disease-associated LCRs.

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Visualization of Human multivariate genomic data via sonic modeling. *G. Wyckoff[1], A. Solidar[1], M. Brommelsiek[2], J. Batzner[3], D. McIntire[3], P. Rudy[3].* 1) Mol. Biol. & Biochem; 2) Center for Creative Studies; 3) Conservatory, U. Missouri-Kansas City, Kansas City, MO.

A common problem in the analysis of large genomic data is the visualization of the data in a meaningful, information-rich way. Most approaches attempt to simplify specific components of multivariate data to compress the portrayed information into one or more 2D figures. At worst, data is simply presented in tabular format, which is difficult to visually extract information from. The Genetic Auralization Project (GAP) explores a novel method of data presentation utilizing sound. Sound and music can portray complex data in a straightforward, meaningful, and intuitive way. This sort of sonic modeling has to the best of our knowledge never been utilized for large scale biological data(1). We employ this methodology to examine evolutionary rate information generated from our analysis of human patterns of substitution, chromosomal location, and expression(2,3). We identified several categories of genes, including genes with high Ka/Ks, genes with strong conservation in regions of moderate evolutionary rate, positively selected genes with narrow expression, and genes with a small domain of rapid evolution). We utilized the GAP prototype to screen through genes in each category. Genes with significantly aberrant patterns (>2 S.D from mean values), were detected at significantly high rates ($p<0.01$ versus random gene assignmen) in all examined classes. While our interests are in the detection of positive selection and identification of evolutionary patterns in primates, the techniques we present here can be adapted to work with many of the types of complex data generated by datamining and genomic studies within humans and related species. We argue that sonic modeling, or auralization, can increase the utility and precision of data visualization for human genomic studies. 1. Bregman, A, S, Auditory Scene Analysis: Cambridge, Mass: The MIT Press, 1990.

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SNPdetector: a Software Tool for Sensitive and Accurate Detection of Single Nucleotide Polymorphisms and mutations in fluorescence-based resequencing. *J. Zhang[1], D. Wheeler[2], I. Yakub[2], S. Wei[2], R. Sood[3], W. Rowe[1], P. Liu[3], R. Gibbs[2], K. Buetow[1].* 1) Lab Population Genetics, NCI, Bethesda, MD; 2) Human Genome Sequencing Center & Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Genetics and Molecular Biology Branch, NHGRI, Bethesda, MD.

Identification of Single Nucleotide Polymorphisms (SNPs) and mutations is important for the discovery of genetic predisposition to complex diseases. PCR re-sequencing is the method of choice for de novo SNP discovery. However, manual data analysis has been a major bottleneck for its application in high-throughput screening due to lack of a sensitive and accurate computational method for automated SNP detection. We developed a software tool, SNPdetector, for automated identification of SNPs and mutations by fluorescence-based resequencing. SNPdetector was designed to model the process of human visual inspection and has a very low false positive and false negative rate. We demonstrate the superior performance of SNPdetector in SNP and mutation analysis by comparing its results with those derived by human inspection, a popular SNP detection tool polyphred, and independent genotype assays in three large-scale investigations. The first study identifies and validates inter- and intra-subspecies variations in 4,650 traces of 25 inbred mouse strains that belong to either the Mus musculus species or the Mus spretus species. Unexpected heterozgyosity in Cast/Ei strain was found in two out of 1,167 mouse SNPs. The second study identifies novel SNPs in 133,440 traces in four ENCODE regions of the human genome (ENCODE Consortium, 2004). The third study detects ENU-induced mutations (at 0.04% allele frequency) in 64,896 traces of 1,236 zebra fish. The three large and diverse test data sets analyzed in this study demonstrate that SNPdetector is an effective tool not only for genome-scale research investigation but also for clinical studies involving a large sample. SNPdetector runs on Unix/Linux platform and is available publicly (http://lpg.nci.nih.gov).

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Possible founder mutation for juvenile hyaline fibromatosis in Brazil. *M.D. Ramaswami[1], P.S. Hart[2], A.C. Acevedo[3], D. Pallos[4], T.C. Hart[1].* 1) Section on Craniofacial & Dental Genetics, NIDCR, Bethesda, MD; 2) Office of the Clinical Director, NHGRI, Bethesda MD; 3) University of Brasilia Brasilia, Brazil; 4) School of Dentistry, University of Taubate, Taubate, Brazil.

Juvenile hyaline fibromatosis (JHF) and infantile systemic hyalinosis (ISH) are allelic autosomal recessive disorders caused by mutations in the anthrax toxin receptor 2 (*ANTXR2*) gene, also known as the capillary morphogenesis protein 2 (*CMG2*) gene. JHF and ISH share many clinical similarities, including papulonodular skin lesions, gingival hypertrophy, and flexion contractures, but ISH usually has an earlier age at onset, a more painful and severe course, and often death in infancy from multisystem failure. A total of 18 different mutations have been described in 22 families with JHF or ISH. Of these mutations, 3 have been found in two kindreds and 1 (c.1073_1074insC) has been found in five kindreds. We have recently ascertained three unrelated Brazilian families segregating JHF, two of which were known to be consanguineous. PCR amplification and direct sequencing of all the *ANTXR2* exons revealed that all affecteds were homozygous for the same mutation: a single nucleotide deletion in exon 13 (c.1074delT). This mutation causes a frameshift and introduces a premature truncation site (p.K361fsX408). Haplotype analysis of intra- and intergenic polymorphisms suggests the presence of a common ancestor for all three families. This mutation has been previously reported in a Kuwaiti family with ISH. The difference in phenotype may be related to ethnic differences, modifying genes or to polymorphisms within the *ANTXR2* gene. The c.1074 region may represent a mutational hotspot as 3 of the 18 known mutations occur at this region.

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Molecular evolution of Ataxin-1 CAG repeat in primates. *T. Kurosaki, A. Ninokata, S. Ueda.* Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

Spinocerebellar ataxia type 1 (SCA1) is a dominant neurodegenerative disorder characterized by degeneration of cerebellar Purkinje cell and neurons within brain stem and spinal cord. Expression of unstable translated CAG repeats that encodes a poly-glutamine tract in coding region of Ataxin1 has been implicated as a cause of the neuronal disorder. The repeat range of a poly-glutamine tract in normal individuals is 6-44, whereas mutant protein carries 39-82 repeat. It has been controversial whether the poly-glutamine tract of Ataxin1 innately has a molecular function or not, whereas poly-glutamine binding protein 1 (PQBP1) was recently reported to bind the tract and was involved in the pathology of SCA1. Interestingly, the poly-glutamine tract is absent in murine Ataxin1 homologue. To make clear an evolutionary history of the poly-glutamine tract in primates, we determined the repeat regions of 25 primate species (2 prosimians, 7 New World monkeys, 12 Old World monkeys and 4 apes). The repeats of prosimians and New World monkeys did not compose homopolymeric state in which they were interrupted with proline cording CCRs. Prosimians and New World monkeys examined in this study were homozygous, whereas 5 in 12 Old World monkeys and 2 in 4 apes were heterozygous. All of the Old World monkeys had uninterrupted CAG repeat whereas apes and human fundamentally contained interruptions. While CAT interruptions are observed in orangutan, gorilla, chimpanzee and human, not CAT but CAA was found in gibbons. This indicates that the nucleotide substitution on the third position of CAG (G to A/G to T) was occurred twice or more in the hominid lineage. Taking into account that the uninterrupted CAG repeat tends to increase the length variability and almost all SCA1 patients contain uninterrupted CAG repeat, the nucleotide interruption makes a contribution to lineage specific polymorphism and might be playing a role for the repeat stability.

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A variant of the gene encoding Leukotriene A4 Hydrolase confers ethnic specific risk of myocardial infarction. *A. Helgadottir[1], A. Manolescu[1], A. Helgason[1], G. Thorleifsson[1], U. Thorsteinsdottir[1], D. Gudbjartsson[1], S. Gretarsdottir[1], K.P. Magnusson[1], A. Hicks[1], S.F.A. Grant[1], A.I. Levey[2], V. Gudnason[3], A.A. Quyyumi[2], E.J. Topol[4], D.J. Rader[5], J. Gulcher[1], H. Hakonarson[1], A. Kong[1], K. Stefansson[1].* 1) DeCode Genetics, Reykjavik, Iceland; 2) Emory University School of Medicine, Atlanta, GA; 3) Icelandic Heart Association, Reykjavik, Iceland; 4) Cleveland Clinic Foundation, Cleveland, OH; 5) University of Pennsylvania School of Medicine, Philadelphia, PA.

We recently reported that variants of the gene encoding 5-lipoxygenase activating protein (ALOX5AP/FLAP), a member of the leukotriene pathway, confer risk of myocardial infarction (MI) and stroke. Here we report the association of a variant of another gene in the same pathway, leukotriene A4 hydrolase (LTA4H) to MI. In an Icelandic cohort, a haplotype (HapK) across the LTA4H confers modest risk of MI, especially of MI with additional atherosclerotic manifestations (P=0.009, RR = 1.45). In addition, we show that HapK is positively correlated with the production of leukotriene B4 (P=0.01), the main product of the enzyme encoded by LTA4H, suggesting that the risk is mediated through an upregulation of the leukotriene pathway. In a study of 3 large cohorts from the United States the association of HapK to MI was replicated, both in European Americans (P=0.019, RR=1.16) and in African Americans (P=0.000022, RR=3.57). Notably, the observed risk in the African Americans is significantly greater (P < 0.001) than in individuals of European ancestry. About 27% of European American controls carried at least one copy of HapK, compared to only 6% of African American controls. Our analysis indicates that HapK is rare in Africa, suggesting that the occurrence of HapK in African Americans is primarily due to admixture. In support of this notion we demonstrate that African American carriers of HapK have, on average, more European ancestry, than African Americans who do not carry HapK (P = 0.0007), although this excess is not sufficient to explain the observed association between HapK and MI.

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Functional polymorphism in Secretogranin II associates with human essential hypertension in African-American population. *G. Wen[1], J. Wessel[1], S.K. Mahata[1], M. Mahata[1], N.R. Mahapatra[1], F. Rao[1], M. Stridsberg[2], D.W. Smith[1], P. Mahboubi[1], N.J. Schork[1], D.T. OConnor[1], B.A. Hamilton[1].* 1) Dept Medicine, Univ California, San Diego, La Jolla, CA; 2) the Department of Medical Sciences, University Hospital, Uppsala, Sweden.

Secretogranin II (SCG2) is a member of the chromogranin/secretogranin, which is costored and coreleased with catecholamines from secretory vesicles. To find polymorphisms that could alter SCG2 function we resequenced the entire gene and proximal promoter region with ~6.5 kb footprint in total from 180 ethnically diverse human subjects. We identified 35 SNPs and one mono-repeat polymorphism in proximal promoter. Only four SNPs have minor allele frequencies greater than 5% in the general population. However, by subgroups, the African-American population has as many as 10 common SNPs, Asian and Hispanic populations only have one common SNP, and the Caucasian population does not have any common SNPs. The most common SNP of SCG2 in general population, SNP_736, appeared common only in African-American samples. The nucleotide diversity value (=0.000203) across the entire gene in general population is several folds lower than average values for several loci examined in the same samples. Three SNPs whose minor alleles are ancestral (shared with chimp) are located in a conserved intron region. To ask whether these polymorphisms are associated with plasma secretoneurin and hypertension we resequenced more subjects (383 in tortal) in African American population and found that significantly associates with secetoneurin (SNP_964, P=0.016) and hypertension (SNP_736, P=0.0046) for two of these SNPs. Admixture analysis from 33 loci across human genome ruled out the possibility of population stratification (X^2=27.83, P=0.7223. Standard neutrality tests and haplotype network suggests positive selection in this locus.Reporter gene assays showed A allele was able to enhance gene activity significantly compared with G allele in transfected cells. In conclusion, we found a functional polymorphism that associates with human hypertension in African-American population and positive selection might be a driving force in shaping its distribution.

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simuPOP: a forward-time population genetics simulation environment. *B. Peng, M. Kimmel.* Dept Statistics, Rice Univ, Houston, TX.

simuPOP is a forward-time population genetics simulation environment. The core of simuPOP is a scripting language (Python) that provides a large number of objects and functions to manipulate populations, and a mechanism to evolve populations forward in time. Using this R/Splus-like environment, users can create, manipulate and evolve populations interactively, or write a script and run it as a batch file. Due to its flexible and extensible design, simuPOP can simulate large and complex evolutionary processes with ease. At a more user-friendly level, simuPOP provides an increasing number of built-in scripts that perform simulations ranging from basic population genetics models to generating datasets under complex evolutionary scenarios.

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Use of the Norfolk Island Genetic Isolate to Identify Genetic Risk Factors for CVD. *L.R. Griffiths[1], C. Bellis[1], R.A. Lea[1], R. Hughes[1], S. Quinlan[1], S. Heath[2], J. Blangero[3].* 1) Genomics Research Centre, School of Medical Science, Griffith Uni Gold Coast, Australia; 2) Centre National de Génotypage, Crémiuex, Evry, France; 3) Department of Genetics, Southwest Foundation for Biomedical Research, Texas, US.

Geographical isolation and limited environmental variation make genetic isolates powerful tools for gene mapping. Our research is aimed at identifying the genes that play a role in cardiovascular disease (CVD) risk and the Norfolk Population may be of significant use in these endeavours. Norfolk Islanders are primarily descended from 18th Century English (Bounty) sailors and Polynesian women. There have strong family groupings and well-documented family histories, providing unique characteristics for a genomic investigation into complex disease. We have recruited individuals from this isolate, to investigate the genes involved in CVD. DNA samples from two-thirds of the Islands adult permanent population have been prepared for these studies. 602 individuals have been collected with information and phenotypes relating to risk of cardiovascular disease including blood pressure, cholesterol, triglyceride, BMI, exercise, smoking levels and diet. Most of these individuals fit within a single large, 12-generational (~6500 individuals) pedigree extending back to the original founders. Heritability and power estimates indicate that this population should be particularly useful for identifying QTL that relate to CVD risk. We conclude that the pedigree should provide a unique and powerful resource for complex disease gene mapping and we are currently investigating implicated genomic loci.

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Effects of B Group Vitamins and the MTHFR C677T Polymorphism on Carotid Intima-Media Thickness in Normal Population. *W.L Cheng[1], H.W Chen[2], H.C Chiang[2], C.S Huang[1], C.L Kuo[1], C.S Liu[1,2,3].* 1) Vascular & Genomic Res, Changhua Christian Hosp, Changhua, Taiwan; 2) Institute of Nutritional Science, Chung Shan Medical University, Taichung, Taiwan; 3) Department of Neurology, Changhua Christian Hospital, Changhua, Taiwan.

Methylenetetrahydrofolate reductase (MTHFR) genotype; concentrations of folate, vitamin B6, and vitamin B12; and age were significantly correlated to the plasma homocysteine (Hcy) concentration ($P < 0.05$). With stratification by age and B vitamin concentrations, 3 risk factors were found to enhance the effect of the MTHFR 677TT genotype on the increase in carotid IMT: age > 50 y, plasma folate < 8.3 mg/dL, and vitamin B12 < 618 pg/mL ($P < 0.05$). A similar effect of the MTHFR 677TT genotype on the increase in plasma Hcy was also found when the subjects were stratified by age, plasma folate, and plasma vitamin B12. Age, folate, and vitamin B12 may be 3 determinant factors in predicting the MTHFR 677TT related carotid atherosclerosis.

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New dbSNP resources to support genotype and haplotype submissions. *S.T. Sherry.* NCBI, National Library of Medicine, National Institutes of Health, Bethesda, MD.

The growth and acquisition strategy for dbSNPs current inventory of 26.2 million SNPs for 25 species will be discussed in the context of the assay sources, validation rules, and placement filters used to establish variation annotation on genome assemblies. Specific details will be provided for the filtering rules used to provide 8.2 million human SNPs to the International HapMap project as potential targets for genotype selection. The presentation will cover new dbSNP resources for visualizing and downloading genotype and frequency data, enhanced programmatic access to dbSNP via NCBI eUtil services, and conclude with the the current status of haplotype representation and content in dbSNP. Details of the haplotype data model, submission requirements, and accessioning scheme will be presented for comment.

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ALFRED - The ALlele FREquency Database - an update. *S. Stein[1], H. Rajeevan[2], U. Soundararajan[1], K.-H. Cheung[2], J.R. Kidd[1], A.J. Pakstis[1], R. Gadagkar[2], P.L. Miller[2], K.K. Kidd[1].* 1) Department of Genetics, Yale University School of Medicine, CT 06520-8005, USA; 2) Center for Medical Informatics, Yale University School of Medicine, New Haven, CT 06520-8005, USA.

ALFRED (http://alfred.med.yale.edu) is an actively curated database designed to make allele frequency data on anthropologically defined human population samples readily available to the scientific community. The database continues to expand and now contains over 30,000 allele frequency tables. Many research questions involve interpreting allele frequencies in a geographic context. ALFREDs newest feature is the implementation of a prototype web-based geographic map interface using GIS (Geographic Information System). Populations are located graphically on a world map and detailed information on them is retrieved by clicking on the points displayed. This interactive map can also display pie charts to represent allele frequencies. When a polymorphism is selected, pie charts depicting allele frequencies across populations are displayed on the world map. A more global view displays a pie chart (frequencies averaged across samples within a population) at the location of each population having data on that site. The interface can also provide a detailed view showing allele frequencies as pie charts for each sample within a selected population. Anthropologic genetics is plagued by an empty matrix problem: the paucity of markers uniformly typed on a large number of populations. This presents a problem for those in population genetics who want to do an integrated analysis. ALFRED can be used to counter this problem. A survey of data assembled in ALFRED shows that only approximately 2 dozen markers have been uniformly studied on the same 100+ populations. Solely by virtue of their having already been typed on multiple populations, these 2 dozen markers became a logical initial set for use in new populations to maximize global comparability. ALFRED is supported by the U. S. NSF (BCS0096588).

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dbRIP: a Database of Retrotransposon Insertion Polymorphisms in Human. *P. Liang[1], J. Wang[1], L. Song[1], D. Grover[2], S. Azrak[1], M.A. Batzer[1].* 1) Dept of Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) Dept of Biological Sciences, Louisiana State Univ, Baton Rouge, LA.

*Alu*s and L1s are the two major types of active retrotransposable elements in the human genome with ~1 million and half million members, respectively, constituting over 30% of the genome. These elements play important roles in the genome and generate a unique and significant part of genome diversity among humans through their recent and ongoing integrations. Polymorphic (for presence or absence) retrotransposon insertions are very useful genetic markers in population genetics studies as being identical-by-descent and free of homoplasy. We have constructed, dbRIP, a database of Retrotransposon Insertion Polymorphism (RIPs) to document all known human genetic polymorphisms derived from retrotransposon insertion. Currently, dbRIP contains a non-redundant list of 1598 and 560 polymorphic *Alu*s and L1s, respectively, and it is our plan to expand dbRIP in future to include other types of retrotransposons, such as SVA. In dbRIP, we deploy the utilities of the genome browser developed at the University of California at Santa Cruise for user-friendly querying and browsing of RIPs along with all other genome information. Users can query RIPs by genetic IDs (gene IDs, accessions, STS, etc), chromosome locations, and DNA or protein sequences using the utilities of the genome browser. A special query interface is also implemented to allow search of RIPs by ethnic group, allele frequency, RIP IDs, subfamily, gene context, disease association, and author. For each RIP, user have access to the detailed RIP information including sequences (flanking genomic regions, TSDs, and retrotransposon), classification, primers, PCR conditions, genotype data, known associated disease, and publications describing the polymorphism. dbRIP is accessed at http://falcon.roswellpark.o rg:9090/, and the entire RIP data set is available for downloading. This research is in part supported by NCI grants CA16056 (RPCI), CA101515 (PL), and GM59290 (MAB), by NSF grant BCS-0218338 (MAB), by Roswell Alliance Developmental Fund (PL), and by the Louisiana Board of Regents Millennium Trust Health Excellence Fund (MAB).

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The Singapore human polymorphism/mutation database: a country-specific database for mutations and polymorphisms in inherited disorders and candidate gene association studies. *E.C. Tan[1], M. Loh[2], D. Chuon[2], Y.P. Lim[2].* 1) Defense Medical Env Res Inst, DMERI@DSO, Kent Ridge, Singapore; 2) Bioinformatics Institute, Republic of Singapore.

There is good evidence for population-specific mutations for single gene disorders and ethnic difference in the frequencies of genetic variations involved in complex disorders. The Singapore Human Polymorphism/Mutation Database is thus created to provide clinicians and scientists access to a central genetic database for the Singapore population. The data catalogued include mutations identified for Mendelian diseases, and frequencies of polymorphisms which had been investigated in either healthy controls or samples associated with specific phenotypes. Data from journal articles are identified by searching through PubMed, other online resources, and via personal communication with researchers. The relevant articles used come from 63 different journals dated from 1972 to 2005. There are also direct submissions of unpublished data from researchers. The total number of polymorphisms/mutations included in the database is currently 298 for 104 genes, which are categorized alphabetically and also searchable by name and disease. Information captured for each variant of the gene includes protein encoded, phenotype associated, gender, size and ethnic origin of the samples, as well as genotype distribution and allele frequencies for each group. There are also direct links to OMIM and the corresponding abstracts on PubMed. With online submission and frequent update from PubMed searches, the database will be kept current so it will be an important resource for academic and clinical users to facilitate molecular diagnosis of Mendelian disorders and improve study designs for complex traits. It will be useful not only to researchers in Singapore, but also countries with similar ethnic background such as China, Taiwan, Hong Kong, Indonesia and Malaysia. Besides Southeast Asian countries, western countries such as the United States, Canada and the United Kingdom which have significant number of immigrants with similar ethnic origin will also find the database useful for patients from such ethnic origin.

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African American mitochondrial DNAs are likley to match common African mtDNA haplotypes. *B. Ely[1], J.L. Wilson[2], B.A. Jackson[2].* 1) Department Biological Sciences, University of South Carolina, Columbia, SC; 2) Department of Work Environment, University of Massachusetts, Lowell, MA.

Mitochondrial DNA (mtDNA) haplotypes are useful tools for tracing maternal ancestry. Numerous studies have demonstrated that human mtDNA haplotypes can be used with confidence to identify the continent where the haplotype originated. Ideally, mtDNA haplotypes also could be used to identify a particular country or ethnic group from which the maternal ancestor emanated. However, the geographic distribution of mtDNA haplotypes is greatly influenced by the movement of both individuals and population groups. Furthermore, mtDNA haplotypes are passed from one ethnic group to another by intermarriage. As a consequence, common mtDNA haplotypes are shared among multiple ethnic groups. We have been studying the distribution of mtDNA haplotypes among West African ethnic groups to determine whether mtDNA haplotypes can be used to reconnect blacks of the Americas and the Caribbean to a country or ethnic group of a maternal African ancestor. The nucleotide sequence of the mtDNA human variable region I (HV-1) usually provides sufficient information to assign a particular mtDNA to the proper haplogroup, and it contains most of the variation that is available to distinguish a particular mtDNA haplotype from closely related haplotypes. In this study, samples of African American and Gullah HV-1 haplotypes were compared to a database of 3200 HV-1 haplotypes from sub-Saharan Africa and the incidence of perfect matches was recorded for each sample. We found that more than 40% of the sampled haplotypes exactly matched common haplotypes that were shared among multiple African ethnic groups. Another large fraction did not match any sequence in the database, and fewer than 15% were a perfect match to a single African ethnic group. Thus, the most common African haplotypes found in America are exact matches to the most common haplotypes found in sub-Saharan Africa. These results indicate that mtDNA sequence information is unlikely to provide sufficient information by itself to conclusively link most individual African American mtDNAs to either a single ethnic group or a particular country in Africa.

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No association between gr/gr deletions and infertility in Brazilian males. *S.D.J. Pena[1], C.M.B. Carvalho[1], L.W. Zuccherato[1], L. Bastos-Rodrigues[1], F.R. Santos[2].* 1) Dept. Biochemistry, UFMG, Belo Horizonte-MG, Brazil; 2) Dept. General Biology, UFMG, Belo-Horizonte-MG, Brazil.

The Y chromosome carries several spermatogenesis genes distributed in three regions: AZFa (azoospermia factor a), AZFb, and AZFc. Microdeletions in these regions have been seen in 10-15% of sterile males with azoospermia or oligozoospermia, the most frequent of them being complete deletion of AZFc region (3.5 Mb). Sequencing of AZFc showed a highly repetitive structure with several palindromic segments, thus explaining the high frequency of rearrangements in this region. A partial AZFc deletion named gr/gr, which removes only some gene copies, has been singled out as a risk factor for spermatogenic failure. However, other authors disagree and prefer to consider it as of no clinical relevance. We then decided to investigate the association of gr/gr deletions and infertility in Brazilian males. We analyzed 357 individuals (117 azoospermic, 122 fertile and 118 of unknown, presumably normal, fertility) with the following STSs: sY1161, sY1191, sY1201, sY1206, sY1258 and sY1291. We observed 12 gr/gr deletions: five in infertile men (4.3%), three among fertile males (2.5%) and four in individuals of unknown fertility (3.4%). These differences were not statistically significant. We then decided to ascertain whether the clinical impact of the gr/gr deletion was associated with the Y chromosome genetic background. We typed Y chromosome haplogroups using 23 unique event polymorphisms (UEPs). We found that the gr/gr deletions occurred in six different Y chromosome backgrounds, suggesting that it occurred recurrently at least six different times. The haplogroups found were R, K* (xMNOP), F*(xJ), E1, E3b2, E3b*, all common in white Brazilian males. There was no single haplogroup associated with infertility. Taken together, these results show a lack of association between the occurrence of gr/gr deletions and the infertile phenotype in Brazilian men.

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A3243G and A12026G variants in mitochondrial DNA of Diabetes Mellitus Type 2 Mexican Patients. *J.M. Oliva[1,2], A. Sarralde[2], J.M. Escalante-Pulido[2], O.A. Rivera-Martinez[1], I.P. Davalos[1,2], L.E. Figuera[1,2], L. Sandoval[1,2].*
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Mitochondrial (mtDNA) variants have been associated to Diabetes Mellitus type 2 (DM). Previous studies in Japanese population showed an association between variants A3243G, A12026G in mtDNA and DM2. A3243G variant in the tRNA gene MT-TL1 is the most common with a prevalence of 1-4 % in Asian population, this mutation is involved in the synthesis of tRNA leu(UUR) and the transcription terminal factor. The variant A12026G prevalence in DM2 patients is of 3.95% (10 of 253) in Japanese DM2 patients, it is located in the MT-ND4 gene in the complex I mitochondrial. Objective: Identify A3243G and A12026G variants in mtDNA of DM2 Mexican patients. Methods: 200 DM2 DNA samples were analyzed to identify mtDNA-A12026G and A3243G variants by PCR/RFLP`s (ApaI and HincII respectively). Results: A3243G mutation frequency was 2% (2/200) and A12026G variant was no present. Conclusion: A12026G variant is not present in the study population and A3243G mutation frequency in DM2 Mexican population was similar to other population studies in DM2 patients.

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Evolution of DSCAM and its related genes. *K. Onishi[1], S. Ueda[2].* 1) Biosciences Information Research, Research Information Research Division, National Institute of Informatics 2-1-2 Hitotsubashi, Chiyoda-ku, Tokyo 101-8430, Japan; 2) Dept. of Biological Science, Graduate School of Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

Human DSCAM (Down syndrome cell adhesion molecule) and its paralogue, DSCAML1, express mainly in central nervous system and have characteristic domain structures: nine immunoglobin (Ig) domains, four fibronectin type III (FN III) domains, one Ig domain, and two FN III domains, in this order (Ig(9)-FNIII(4)-Ig(1)-FNIII(2)). Their putative orthologous genes also exist in insects (but not in nematode), which have four alternative exon clusters and can generate as many as tens of thousands of splicing isoforms. Both of them have the same domain structures, however, their orthologous relationships between vertebrates and invertebrates remain unclear. Here, we constructed phylogenetic trees based on each of the two domains (Ig and FN III) and found that they indeed are orthologous. Sax-3/Robo genes of vertebrates and invertebrates (including nematode) are homologous to the DSCAM genes and have both Ig and FN III domains (Ig(5)-FNIII(3)). We also analyzed the evolution of genomic structures of the two gene structures (DSCAM and Sax-3/Robo) from the viewpoint of their domain structures. Our results strongly suggest that both DSCAM and Sax-3/Robo genes exist in the common ancestor of vertebrates and invertebrates, and that their genomic structures, as well as their amino acid sequences, are highly conserved in vertebrates.

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Genetic analysis of Y, mtDNA, and STR variation in castes populations of Tamil Nadu, India. *W.S. Watkins[1], B. Mowry[2], H. Smith[2], E. Marchani[1], L. Zhou[3], S. Tirupati[4], R. Thara[4], R. Padmavati[4], J. Sujit[4], K. Soundari[4], L.B. Jorde[1].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Queensland Centre for Mental Health Research, Wacol, Brisbane, Australia; 3) Department of Pathology, University of Utah, Salt Lake City, UT; 4) Schizophrenia Research Foundation India, Chennai, Tamil Nadu, India.

To test the hypothesis that caste populations of the Indian subcontinent are genetically structured, we analyzed 181 Y haplotypes from 4 caste populations living in Tamil Nadu, India. Using 15 polymorphic lineage-defining Y chromosome markers, we demonstrate that Y genetic distances are positively correlated with differences in caste rank (rho = 0.93, $p < 0.01$). R lineages, dominated by the Eurasian R1a, and central Asian/Indian R2 haplogroups, occur at frequencies of 0.5 in 2 Brahmin groups (high status), 0.31 in Mudaliars (middle status) and 0.22 in Dalits (lower status). Thus, this major Y lineage demonstrates a negative correlation with differences in caste status (rho = -1.0, $p < 0.01$). In contrast, the H lineage, which is largely restricted to India and is thought to have originated there, occurs at high frequency in the Dalit population (0.49) but is found at < 0.18 in higher status groups. Genetic distances derived from mitochondrial HV1 sequences or 45 autosomal STRs are lowest for Tamil-speaking Brahmins and Europeans. Exclusion of the linguistically distinct non-Tamil speaking Brahmin group yielded positive correlations between genetic distance and caste rank for STR and mtDNA (rho = 0.87, $p > 0.3$; rho = 0.87, $p > 0.3$). We have observed a similar correlation in caste populations from the neighboring state of Andhra Pradesh. Between-group differentiation for Y markers is greater than that found for mtDNA or STRs (F_{ST}/R_{ST}: 0.061, 0.046, and 0.007, respectively). These data are consistent with a structured, historical influx of Eurasian male lineages into the caste system.

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Y Chromosome Evidence of Southern Origin of the East Asian Specific Haplogroup O3-M122. *H. Shi[1,2,6], Y. Dong[3], B. Wen[4], C. Xiao[3], P.A. Underhill[5], P. Shen[5], R. Chakraborty[7], L. Jin[4,7], B. Su[1,2,7].* 1) Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoolgy, Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Kunming Primate Research Center, Chinese Academy of Sciences, Kunming, Yunnan, China; 3) Key Laboratory of Bio-resources Conservation and Utilization & Human Genetics Center, Yunnan University, Kunming, Yunnan, China; 4) State Key Laboratory of Genetic Engineering and Center for Anthropological Studies, School of Life Sciences, Fudan University, Shanghai, China; 5) Department of Genetics, Stanford University, CA; 6) Graduate School of Chinese Academy of Science, Beijing, China; 7) Center for Genome Information, University of Cincinnati, OH.

The prehistoric peopling of modern humans in East Asia remains controversial in view of the early population migrations. Here we presented a systematic sampling and genetic screening of an East Asian specific Y chromosome haplogroup (O3-M122) in 2,332 individuals from diverse East Asian populations. Our results indicated that the O3-M122 lineage is dominant in East Asian populations with an average frequency of 44. 3 %. The microsatellite data showed that the O3-M122 haplotypes in southern East Asia is more diverse than those in northern East Asia, suggesting a southern origin of the O3-M122 mutation. It was estimated that the early northward migration of the O3-M122 lineages in East Asia occurred about 25,000-30,000 years ago, consistent with the fossil records of modern humans in East Asia.

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Lack of Evidence for Balancing Selection on PRNP M129V in Chinese. *Q. Zan[1], B. Wen[1], Y. He[1], Y. Wang[1], S. Xu[1], J. Qian[1], D. Lu[1], L. Jin[1,2].* 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Center for Genome Information, Fudan University, Shanghai, China.

The M129V in PRNP is the primary susceptible site to prion diseases such as kuru. It has been shown that balancing selection played a critical role in shaping the distribution of 129V in worldwide populations. However, the statistic evidence in Japanese was substantially weaker, possibly due to a lower level of variation in PRNP gene in East Asians. We therefore surveyed the frequency of 129V in 10 Han Chinese populations and examined the role of balancing selection on the PRNP gene by sequencing a substantially larger genomic segment (15kb) which encompasses the entire genomic region of the PRNP gene. We showed that the balancing selection that exerted on 129V in the other parts of the world was less likely to have played a significant role in East Asians.

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Lactase persistence in East Africans and Bedouins is not associated with the extended haplotype found in Europeans. *C.J.E. Ingram[1], M. Elamin[1,2], C.A. Mulcare[1], M.E. Weale[1], M.G. Thomas[1], N. Bradman[1], D.M. Swallow[1].*
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Background Lactase, the intestinal enzyme responsible for digestion of lactose in milk is downregulated in mammals after weaning. However in humans, lactase persistence (expression of the enzyme in adulthood) is a polymorphic trait. It has been shown in Europeans that lactase persistence is controlled by a regulatory element, *cis*-acting to the lactase gene *LCT*, and is located within a region of strong linkage disequilibrium, with only 3 common haplotypes. One of these haplotypes, designated **A**, forms the core of an extended (500kb+) haplotype, which is associated with lactase persistence and is at high frequency, probably due to the effect of selection for this trait (Poulter et al, 2003 Ann Hum Genet 67:298, Bersaglieri et al 2004, Am J Hum Genet 74:1111). However the single SNP (-13.9kb*T) proposed to be causative of lactase persistence (Enattah et al 2002 Nat Genet 30:233) was absent in several groups in sub-Saharan Africa, in which the trait is common (Mulcare et al 2004 Am J Hum Genet 74:1102). **Aims** To examine the association of allele and haplotype frequencies with lactase persistence in populations of non-European ancestry. **Methods** We studied 3 African groups where lactase persistence is prevalent; pastoralist Fulani from Cameroon (n=74), Beni-Amer (n=100) and Jaali from Sudan (n=91), and three Middle Eastern Bedouin groups (n=86) as well as neighbouring Arabs. For the Africans we collected milk drinking data, and in one group, lactose tolerance data. We tested C-13.9kbT, and 4 other SNPs that discriminate the core LCT haplotypes previously found in Europeans and Africans. **Results** The presence of -13.9kb*T was confirmed in the Fulani (0.367), and was in most cases on an **A** haplotype chromosome. The allele and haplotype frequencies in the Beni-Amer, Jaali and Bedouins were very different; -13.9kb*T was very rare (0.005-0.02), and the core **A** haplotype was not associated with lactase persistence. **Conclusions** The long European *LCT* **A** haplotype is not frequent in East African and Middle Eastern milk drinkers, where there may be another cause of lactase persistence.

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Evolution of the Agouti Signaling Protein Gene (*ASIP*) in Primates: The Loss-of-the *ASIP* in the Gibbons. *K. Nakayama, T. Ishida.* Department of Biological Sciences, Graduate School of Science, The Universtiy of Tokyo, Tokyo, Japan.

Agouti signaling protein is an endogenous antagonist of melanocortin receptors, which control a wide range of physiological functions in humans. The central role in regulation of the melanocortin system implied that agouti signaling protein has been relevant to evolution of various physiological traits in humans and other primate species. In the present study, we have tried to determine DNA sequences of the agouti signaling protein gene (*ASIP*) of various primate species to know molecular evolutionary properties of the *ASIP*. Unexpectedly, we found that the whole coding region of the *ASIP* was missing from the gibbons, which is a large group of ape species from Southeast Asia. This 100kb deletion was mediated by an unequal homologous recombination between *AluSx* elements and apparently occurred prior to the divergence of the present gibbon species. The maximum likelihood analysis for successfully determined *ASIP* sequences from primates and other mammalian species showed that the *ASIP* has been under purifying selection throughout the evolution of the mammals. This suggested that agouti signaling protein has carried important physiological roles, probably in pigmentation and in energy homeostasis. The loss-of-the *ASIP* is considered to have had momentous contributions in the formation of biological characteristics regarding the melanocortin system in the gibbons, although it is still unclear whether a lineage specific relaxation of functional constraints or positive selection was responsible for the fixation of such large deletion during the evolution of the gibbons.

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Molecular Evolution of 5' Flanking Regions of 87 Candidate Genes for Atherosclerotic Cardiovascular Disease. *I. Kullo, K. Ding.* Cardiovascular Dept, Mayo Clinic, Rochester, MN.

Background. Inter-individual variation in quantitative traits as well as in disease susceptibility may be due to differences in the level and spatio-temporal pattern of gene expression. An evolutionary model of genetic variation in cis-regulatory regions may help identify loci of interest in the study of the genetic basis of complex diseases such as atherosclerotic cardiovascular disease (ASCVD).

Methods and Results. We studied the molecular evolution of 87 candidate genes for ASCVD to assess for signatures of selection in 5' flanking regions. Resequenced data for these genes was available in 24 African-Americans, 23 European-Americans, and 1 chimpanzee (Pan troglodytes). Statistical tests of evolutionary neutrality (Tajimas D and Fay & Wus H) were performed using coalescent simulation under a standard neutral model and a population structure model for African-Americans and European-Americans to differentiate selection from human demographic history. Evidence suggestive of selection was present in 5' flanking regions of 4 genes in African-Americans and 7 genes in European-Americans. A modified McDonald-Kreitman test was used to test the neutral theory of molecular evolution in 5' flanking regions, and 10 genes in African-Americans and 11 genes in European-Americans showed a significant excess or deficit of fixed changes over polymorphisms. Haplotype configuration was assessed in 5' flanking regions of genes showing deviation from evolutionary neutrality in either of the above tests. An unusual pattern of haplotypes was noted in 6 genes in African-Americans and 8 genes in European-Americans, providing further evidence for selection in the regulatory regions of these genes.

Conclusion. These results indicate that selection may play a role in establishing variation in 5' flanking regions of a subset of candidate genes for ASCVD and motivate further studies of these loci in determining inter-individual susceptibility to ASCVD.

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The evolution of satellite III DNA subfamilies among primates. *M. Jarmuz, C.D. Glotzbach, K.A. Bailey, L.G. Shaffer.* Health Research and Education Center, Washington State University, Spokane.

Satellite DNA is a dynamic component of eukaryote chromosomes and may play an important role in chromosome structure and karyotypic evolution through the facilitation of exchange events. In our study we analyzed satellite III DNA (satIII DNA) from two subfamilies characterized by their sequence composition: the GGAAT monomer sequence is predominant in the first group, whereas the second has a roughly equal representation of GGAAT and GGAGT motifs. Both groups are present in the short arms of the acrocentric chromosomes and in chromosomes 9 and Y in human and share high homology. We determined the distribution of six subfamilies of satIII DNAthree from group 1 (pTRS-63, pW-1 and pK-1) and three from group 2 (pR-2, pR-4 and pTRS-47)in 25 primate cell lines by PCR with primers specific to each subfamily and sequencing analysis of PCR products. We also determined the presence or absence of the subfamilies in four human reference cell lines and a panel of monochromosomal hybrids of the acrocentric chromosomes. These satellite III subfamilies were not detected in squirrel monkey, african green monkey, baboon, or rhesus monkey. The group 1 subfamily was found only in human cell lines and in corresponding monochromosomal hybrids of the acrocentric chromosomes. The pTRS-47 subfamily was detected in orangutans, gorilla, chimpanzee and bonobo, whereas the pR-2 and pR-4 subfamilies were found in gibbon. Sequence analysis indicated a higher conservation of pTRS-47, than pR-2 and pR-4, for species closely related to humans. In addition, sequence analysis showed that the percentage of sequence divergence between primate and human was higher in pR-2 than pR-4. The presence of pR-2 and pR-4, and absence of pTRS-47 in the gibbon cell lines, indicate that the pR-2 and pR-4 subfamilies are more ancient than pTRS-47. In addition, these findings indicate that the group 2 subfamilies appeared suddenly in gibbon and that they are older than subfamilies from group 1.

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An examination of selection at the molecular level in the forkhead transcription factor gene family. *C.D. Fetterman, M.A. Walter, B. Rannala.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

One manner in which gene families are thought to evolve is through gene duplication and subsequent selection. If one member of a duplicated gene maintains its original function, the other member may mutate and develop a novel function. Positive selection will maintain mutations that enhance fitness while negative selection will eliminate deleterious mutations. To discover the molecular basis underlying the evolution of the disparate members of the forkhead transcription factor gene family, the phylogenetic relationships between genes containing the forkhead domain are being determined to identify residues in forkhead gene family members under strong selection. Nucleotide and protein sequences of genes containing the forkhead domain in all species for which sequence data are available were obtained from the NCBI Conserved Domain database through CDART. A total of 383 unique sequences from 51 different species were chosen for analysis. ClustalX and DIALIGN are being used to align the protein sequences. The MRBAYES program is being utilized to elucidate the phylogenetic relationships among the gene family members. These experiments are yielding a phylogenetic tree of the forkhead gene family. Residues that are targets of strong positive or negative selection are being identified using a codon-based model contained in the PAML program. Genes with residues under strong positive selection may have evolved novel functions and the residues under selection may be key to these new functions. Identifying these sites can help direct future investigations into gene function. Residues under strong negative selection can indicate how genes with different functions diverged from a common ancestor.

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Evolutionary Mechanism of Diversification of Multiple Variable Exons in the Human Genome. *Q. Wu, C. Li.*
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Fifty three neural protocadherin (Pcdh) proteins are encoded by three closely-linked gene clusters on the human chromosome 5 and are expressed in cell-specific patterns in the brain. Two of the three clusters have a striking genomic organization consisting of a tandem array of multiple variable exons and a common set of constant exons, similar to that of the immunoglobulin and T-cell receptor gene clusters. By a genome-wide computational analysis, we found that the human genome contains a large number of genes, including the drug-metabolizing UDP glucuronosyltransferase (UGT1), with multiple variable exons. We performed comprehensive comparative analyses for the vertebrate Pcdh and UGT1 clusters and found that there are species-specific duplications in vertebrate Pcdh and UGT1 genes. Phylogenetic analysis demonstrated that this variable and constant genomic organization is vertebrate-specific and is conserved throughout vertebrate evolution. In addition, comprehensive analysis of the complete repertoire of the closely-related UGT2 clusters in the human, mouse, and rat genomes revealed striking gene conversion and extensive lineage-specific duplication. These results suggest that the tremendous diversity of these clusters is achieved through gene conversion and concerted evolution, in conjunction with the birth-and-death evolution of the duplicated exons and their independent mutations. Moreover, analyzing the pattern of nonsynonymous and synonymous nucleotide substitutions identified codon sites that are likely to have been subject to positive Darwinian selection at the molecular level. These diversified residues of the human Pcdh and UGT proteins likely play an important role in providing the staggering diversity required for neuronal connectivity, chemical detoxification, and drug clearance. These results also suggest that the adaptive natural selection of the specific codon sites play an important role for enhancing the human Pcdh and UGT diversity. This study has interesting implications regarding the evolutionary mechanisms for generating molecular diversity required for neuronal connections in the brain and drug biotransformation in the body.

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High-resolution haplotype analysis in a large European population. *A. Montpetit[1], M. Nelis[2], P. Laflamme[1], X. Ke[3], L. Cardon[3], A. Metspalu[2], T.J. Hudson[1].* 1) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada; 2) IMCB University of Tartu, Estonian Biocentre and The Estonian Genome Project Foundation, Tartu , Estonia; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom.

The HapMap project recently generated genotyping data for more than 1 million SNPs in four populations. The main application of the data is in the selection of SNPs (or tag SNPs) to test in association studies. However, the usefulness of this selection process needs to be verified in populations outside those used for the HapMap project. In addition, it is not known how well the data represents the general population since only 90 to 120 chromosomes were used to build each population's haplotype map and since the genotyped SNPs were selected to have a high minor allele frequency (MAF) in those samples. In this study we analysed more than a thousand individuals from Estonia. The population of this northern European country has been influenced by many different migrations from European and Asian populations. We genotyped 1536 randomly selected SNPs from two 500 kb ENCODE regions on chromosome 2. All these SNPs had been identified through a resequencing study, as part of the HapMap project. We observed that the tag SNPs selected from the CEU sample (derived from U.S. residents with northern and western European ancestry) were very efficient at capturing the variation in the Estonia sample (between 85-90% of the SNPs with a MAF above 2% had an r2 of at least 0.8 with the tag SNPs). An approach using tag SNPs from the combined CEU, CHB and JPT populations was even more effective as it captured about 90-95% of the SNPs (although with an increase of about 30% in the number of tag SNPs used). Using the opposite approach, tags selected from the Estonia samples could almost equally well describe the CEU samples. However, using random data sets of 120 chromosomes to select tag SNPs we show that the sample size used has an important effect on the robustness of the results.

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Neolithic split of R1a1 Y-chromosomes in Northern Eurasia. *M. Derenko[1], B. Malyarchuk[1], G. Denisova[1], M. Wozniak[2].* 1) Institute of Biological Problems of the North, Magadan, Russian Federation; 2) The Nicolaus Copernicus University, Forensic Medicine Institute, Bydgoszcz, Poland.

Y-chromosome variation in a total sample of 1358 males from 14 ethnic groups of Siberia (Altaians-Kizhi, Teleuts, Shors, Tuvinians, Todjins, Tofalars, Sojots, Khakassians, Buryats, Evenks), Central/Eastern Asia (Mongolians and Koreans) and Eastern Europe (Kalmyks and Russians) was studied using 17 SNPs/indel and twelve STR loci. Distribution pattern of Y-chromosomal haplogroups, results on AMOVA and admixture analyses demonstrate the significant differentiation between populations of Eastern Siberia and Altai region. These regional differences can be best explained by different contribution of Central/Eastern Asian and Eastern European paternal lineages into gene pools of modern South Siberians. The populations of Eastern Siberia demonstrate the absolute prevalence of Central/Eastern Asian lineages, whereas in populations of Altai region the highest (70%-98%) paternal contribution resulted from Eastern European descent is revealed. The major component responsible for the high impact of Eastern European lineages into gene pools of Altaians-Kizhi, Shors and Teleuts is R1a1-haplogroup. Our data on Y-chromosome STRs variation demonstrate the clear differences between South Siberian and Eastern European (Russian) R1a1-lineages (with only 6% of haplotypes shared), which can be best explained by apparently ancient than very recent events in South Siberian population history. Despite the haplotypic diversity value of Eastern European R1a1-lineages exceeds that in South Siberia (0.994 0.002 and 0.932 0.018, respectively), the estimated ages for this haplogroup are almost equal in both regional groups - 11,270 4,070 years in Siberia and 11,380 3,200 years in Eastern Europe. These values are very close to the divergence time between two regional groups studied (10,3103,140 years). Moreover, the analysis of R1a1 Y-chromosomes revealed two subclades with distinct, South Altaian and Eastern European, geographic distributions. This work was supported by Russian Foundation for Basic Research (grant 04-04-48746).

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A curious pattern of variation at the ALDH2 locus. *H. Oota[1,2], A.J. Pakstis[1], W.C. Speed[1], J.R. Kidd[1], K.K. Kidd[1].* 1) Dept Genetics, Yale University School of Medicine, New Haven, CT 06520-8005 USA; 2) Dept Integrated Biosciences, Graduate School of Frontier Science, University of Tokyo, Kashiwa, Chiba 277-8581 Japan.

Acetaldehyde dehydrogenase 2 (ALDH2) , encoded by the ALDH2 gene on 12q24.2 in humans, is a key enzyme in ethanol metabolism along with alcohol dehydrogenase (ADH) enzymes. We previously presented LD and haplotype analyses of the ALDH2 region spanning 40 kb and found high LD values across the region, suggesting a lower recombination rate in this region (Oota et al. 2004 Ann. Hum. Gen. 68:93-109). We have extended this study to include more SNPs across 85 kb, encompassing the ALDH2 gene, and have increased the number of populations from 37 to 42. Analyses have revealed 3 distinct regions defined by Fst values that fall into the tails of the Fst distribution: unusually high Fst (95th percentile) at the two ends of the gene and unusually low Fst (5th percentile) across the middle. Each region has high LD with low LD between the regions. The downstream region contains the exon 12 dominant-negative allele that is present only in East Asia and has been postulated to have been subject to positive selection; the markedly different allele and haplotype frequencies in East Asia are responsible for this regions high Fst (0.25-0.36). The Fst values at the upstream end of the gene are as high (0.24-0.38), suggesting selection may also have occurred for this region, but it is the Europeans that have the outlier frequencies in this region. In sharp contrast, the 31 kb region across the middle of ALDH2 has only two common haplotypes averaging 81% and 17% and these account for 98% of the chromosomes in all populations studied with virtually no deviation from this pattern globally (Fst ~0.05 for all SNPs) and the pairwise LD values are very strong (r^2 0.65) in all populations. The similar frequency pattern of these two common haplotypes worldwide is extremely unusual and suggests balancing selection. Supported in part by NIH AA09379.

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Variation in the MBL2 gene and in Native South Americans its relationship to tuberculosis prevalence. *A.K. Wilbur[1], A.M. Hurtado[2], K.R. Hill[2], A.C. Stone[1].* 1) Dept Anthropology, Arizona State University, Tempe, AZ; 2) Department of Anthropology, The University of New Mexico, Albuquerque, NM.

Tuberculosis infection and disease prevalence are especially high in Native South American populations, and among the Aché of Paraguay, exposure to M. tuberculosis is thought to be universal. Our extensive ethnographic and molecular data indicate that prevalence of active disease among the Aché and a neighboring Native group, the Avá, is greater than 30%. Despite extensive evidence that host genetics help determine susceptibility and resistance to tuberculosis in various populations around the world, very little research has been conducted on Native American susceptibility. This project examines the potential interplay of host genetics and environmental factors in tuberculosis susceptibility in Aché and Avá using SNPs at the MBL2 gene, which codes for the mannose-binding lectin (MBL) protein. MBL is a component of both the innate and acquired immune systems, and is particularly important as a nonspecific immune "first responder" before acquired immunity to a pathogen is developed. Three highly variable regions of exon I, previously associated with tuberculosis in other populations, show reduced variability among Aché and Avá. Further, the B allele of codon 54 is associated with TB susceptibility in Aché, and this allele is present in the Aché and Avá at frequencies much higher than those previously reported for any other group in the world. These data are discussed in the context of population history and pathogen encounters.

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Y chromosome haplogroups and AZFc polymorphisms of the Han Chinese in Taiwan. *P. Yen[1,2], Y.W. Lin[1,2], L. Hsu[1], Y.H. Yu[1], T.Y. Hsu[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan.

Han Chinese constitutes 98% of the population in Taiwan. About 85% of them are descendants of early settlers who emigrated from the Southeast coast of China about 400 years ago. To compare with current Han Chinese in China, we randomly selected 580 Han men from the Taiwanese population and determined their Y chromosome haplogroups by genotyping 22 SNP markers, using mostly MALDI-TOF mass spectrometry. We found that 87% of our samples belonged to haplogroup O (containing the M9 and M175 markers), with 61% in the O3 subgroup alone. Other haplogroups found in our samples are C (6%), N (5%), Q (1.2%), R (0.6%), and D (0.3%). The distribution of the various Y haplogroups in our subjects is similar to that found in Han Chinese in Southern China previously reported by other groups. We also determined the prevalence of partial deletions and duplications of the AZFc region on the long arm of the Y chromosome. This region consists entirely of very long direct and invert repeats and is prone to rearrangement. Non-allelic homologous recombination between the gr repeats or between the b2 and b3 repeats removes about half of the AZFc region and generates deletions that are quite common in some populations. We used a novel dose-sensitive PCR assay to determine the copy number of the DAZ genes within AZFc, and applied the plus/minus STS method to study the nature of the partial deletions. The overall frequencies of partial deletion and duplication in our samples were 11% and 1.4%, respectively. Both gr/gr and b2/b3 deletions were present. The frequency of deletion varied significantly among different haplogroups, ranging from 2.3% in O1 to 86% in Q. While gr/gr deletion was found in most haplogroups, b2/b3 deletion was found only in groups C, N, O2 and O3d. Our results show that AZFc partial deletions are common Y polymorphisms in Han Chinese, similar to other populations.

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Genetic similarity of individuals from the same or different human populations. *D. Witherspoon[1], E.E. Marchani[1], C.T. Ostler[1], W.S. Watkins[1], M.D. Shriver[2], D. Ray[3], M.A. Batzer[3], L.B. Jorde[1].* 1) Dept Human Genetics, Univ Utah, Salt Lake City, UT; 2) Dept Anthropology, Pennsylvania State Univ, University Park, PA; 3) Dept Biological Sciences, Louisiana St Univ, Baton Rouge, LA.

Are any two individuals from a particular population as different genetically as any two people from any two populations in the world? The answer depends on the number of loci used to compute genetic distance as well as the choice of populations. We analyzed 75 L1 and 100 Alu insertion polymorphisms genotyped in 246 individuals of European, African, East Asian, and sub-continental Indian ancestry. We computed genetic distances between all possible pairs of individuals in the data set, then determined the frequency, f, with which the distance between random pairs of individuals within continental ancestry groups exceeded the distance between pairs from different groups. With 75 L1 loci, f = 0.20. Adding 100 Alu loci reduces f to 0.15. In a reduced sample including only Indians and Europeans, f = 0.28 with all 175 loci. This is due to the greater similarity of allele frequencies between these two groups relative to the dissimilarity between Africans and Asians (for which f = 0.042.) Theory and the observation of decreasing f with increasing number of loci suggest that even for slightly divergent populations, f should approach zero as the number of loci grows. To test this, we analyzed 11,555 SNP loci typed in a different sample of 248 individuals from the same four continental groups, as well as Puerto Ricans and African-Americans. Consistent with expectations, f = 0.0015 when considering just the four continental groups and 11,555 loci. With the exclusion of the Indian individuals (who are similar to Europeans), f drops to zero. However, including the two admixed populations increases f to 0.058 due to the high degree of overlap between the admixed groups and their ancestral populations (e.g., among Puerto Ricans and Europeans, f = 0.15). Thus while some populations are quite distinct by this measure, among the less divergent groups, some individuals will be more similar to members of other populations than to others in the same population - even with very large sets of markers.

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Frequency of the major susceptibility haplotype for androgenetic alopecia in the Chinese and African American population. *A. Hillmer[1], T. Becker[2], F.F. Brockschmidt[1], S. Hanneken[3], S. Ritzmann[3], J. Freudenberg[4], A. Flaquer[2], C. Metzen[3], P. Propping[4], T. Ruzicka[3], S. Cichon[1], T.F. Wienker[2], R. Kruse[3], M.M. Nöthen[1].* 1) Life & Brain Center, University of Bonn, Bonn, Germany; 2) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; 3) Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany; 4) Institute of Human Genetics, University of Bonn, Bonn, Germany.

Androgenetic alopecia (AGA, male pattern baldness) is the most common form of hair loss. Its pathogenesis is androgen dependent, and genetic predisposition is the major requirement for the phenotype. We recently demonstrated that genetic variability in the androgen receptor gene (*AR*) is the cardinal prerequisite for the development of early-onset AGA, with an etiological fraction of 0.46. Here we compare the haplotype diversity at the *AR* locus of 188 German, 116 African American, and 100 Han Chinese individuals by genotyping 20 genetic variants in a region of 2.5 Mb. The haplotype diversity in the African sample is, as expected, greater than the diversity in the German and Chinese sample. In the German population, the haplotype associated with AGA has a frequency of 0.46. The frequency is 0.07 in African Americans and 0.81 in Han Chinese. The low frequency in the African American sample is in accordance with the postulated lower frequency of AGA in African populations whereas the high frequency of the haplotype in the Chinese sample is in contrast to the generally assumed lower frequency of AGA in Asia.

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Multiplex MIP genotyping of 25,000 non-synonymous SNP's in 270 HapMap samples from 3 ethnic populations and 10 additional non-human primates. *T.A. Cormier, J. Ireland, V.E. Carlton, M. Falkowski, M. Moorhead, K. Tran, P. Hardenbol, A. Erbilgin, R. Fitzgerald, T.D. Willis, M. Faham.* ParAllele Bioscience, SSF, CA.

The HapMap project focuses on a genome-wide approach to define the haplotype block structure. Here we examine the same samples with a focus on genic SNPs, specifically non-synonymous SNPs (nsSNP). This study covered more than 10,000 genes across the genome. The key results show a presence of selection on some nsSNPs, that in non-human primates assay success correlates with evolutionary closeness to humans and gene category, and that there are quantitative copy number variations in some HapMap samples.

Evidence for selection was detected by finding a correlation between decreased allele frequency and likelihood to cause protein damage based on PolyPhen prediction. Additionally, selection was identified by comparison of SNPs in specific Gene Ontology (GO) categories. By comparing different populations and primates, it was possible to identify GO categories where SNPs had shifted allele frequency distributions or shifted assay success rates. As expected, the primate data was also useful to confirm that evolutionary distance from humans was strongly correlated with the success rate of assays. The primate data also identified interesting regions such as 100 contiguous markers across 1.6 Mb of chromosome 11 that failed to genotype in orangutan, possible identifying a region of deletion. Lastly the MIP assay is quantitative and so we used the coverage of SNPs across the entire genome to look for copy number variations. There are several HapMap samples showing signs of megabase size insertions or loss of heterozygosity. Overall this dataset was useful for looking at genome wide events and focusing on SNPs that may have direct biological effects.

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Updating the East Asian mtDNA tree and nomenclature. *Q.-P. Kong[1,2,3], H.-J. Bandelt[4], C. Sun[1,2,3], Y.-G. Yao[1], A. Salas[5], A. Achilli[6], L. Zhong[2], C.-Y. Wang[1,2,3], C.-L. Zhu[1], A. Torroni[6], Y.-P. Zhang[1,2].* 1) Laboratory of Cellular and Molecular Evolution, and Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China; 2) Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming 650091, China; 3) Graduate School of the Chinese Academy of Sciences, Beijing 100039, China; 4) Department of Mathematics, University of Hamburg, 20146 Hamburg, Germany; 5) Unidad de Genética, Instituto de Medicina Legal, Facultad de Medicina, Santiago de Compostela, Galicia, Spain; 6) Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy.

The East Asian mitochondrial DNA (mtDNA) phylogeny has been reconstructed in its main features based on complete sequencing efforts in the past few years. The recently published 672 complete mtDNA sequences from Japan could in principle provide further information to refine and update the East Asian mtDNA tree. Unfortunately, these data bear several shortcomings and the employed haplogroup designations are in conflict with the earlier nomenclature, which may lead to confusion in future phylogenetic and medical studies. Moreover, some less frequent lineages observed in East Asia have not been well characterized so far. To resolve these issues, we selected 19 mtDNAs from our large sample collection for complete sequencing, and to further identify the characteristic mutations of these novel lineages, we adopted motif-search approach in order to distinguish potentially haplogroup-diagnostic mutations of novel lineages from private mutations. By incorporating the previous studies, a total of 989 (nearly) complete mtDNA sequences were (re)analyzed, which led to an updated East Asian haplogroup classification tree that reconciled the conflicts raised among the past studies and standardized the nomenclature for this region.

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Novel sequence variation in the dopamine transporter gene of the cynomolgus macaque. *C. Miller-Butterworth[1], J. Kaplan[2], S. Manuck[1], R. Ferrell[1].* 1) University of Pittsburgh, Pittsburgh PA; 2) Wake Forest University School of Medicine NC.

Social rank and related behaviors (e.g. aggression and impulsivity) have been correlated with differences in central nervous system monoaminergic activity in Old World monkeys, and cerebrospinal fluid (CSF) concentrations of monoamine metabolites and certain behavioral phenotypes (e.g. impulsivity, fear/anxiety) appear to be significantly heritable. Specific genotypes of the human monoaminergic pathways have been linked to personality traits (e.g. extraversion, novelty-seeking) and to CSF monoamine metabolite concentrations. Sequence diversity in monoaminergic gene pathways produces functional variants, which may influence behavior patterns and lead to differences in social rank. This project investigates whether sequence variation in genes of the monoaminergic pathways is significantly associated with behavioral and monoamine metabolite differences previously identified between dominant and subordinate cynomolgus macaques, *Macaca fascicularis*. The dopamine transporter (DAT) was selected as a candidate gene. It regulates synaptic dopamine concentration and duration of dopamine activity through active reuptake of dopamine from the synaptic cleft into the presynaptic terminal. DNA samples were obtained from 500 *M. fascicularis*, for which social rank is known. The complete DAT gene and promoter region were sequenced in 50 randomly selected individuals and screened for novel single nucleotide polymorphisms (SNPs) and other sequence variants. Genotype and allele frequencies were determined for each of the ~80 polymorphisms identified. None of the SNPs correlated with social status. One non-synonymous SNP was identified, which may produce a functional gene variant. It was screened in all 500 individuals by restriction enzyme analysis, but was present in only one heterozygote. None of the macaque SNPs correspond to any identified in the human DAT gene. In contrast to humans, the tandem repeat in the 3 untranslated region of the macaque DAT gene does not vary in repeat number. Furthermore, the macaque gene does not appear to display any significant long range linkage disequilibrium.

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Age of SOD1 A4V mutation causing ALS and founder effect. *M. Saeed[1], H.X. Deng[1], W.Y. Hung[1], N. Siddique[1], L. Dellefave[1], Y. Yang[1], S. Ullah[1], E. Usacheva[1], C. Gelera[2], P. Andersen[2], T. Siddique[1].* 1) Neurology, Northwestern University, Chicago, IL; 2) Department of Clinical Neurosciences, Umea University, Umea, Sweden.

An alanine to valine (A4V) mutation at codon 4 of SOD1 is responsible for 50% of ALS-causing SOD1 mutations in North America. This mutation is rare in Europe. We aimed to estimate the age of the A4V mutation and to search for a founder effect. Fifty-four patients with confirmed A4V mutation and 96 healthy control subjects were genotyped for 21 SNPs across a 21cM region. Also 61 samples with SOD1 mutations other than A4V and 96 sporadic ALS (SALS) cases were genotyped to define the extent of linkage disequilibrium (LD) for the A4V mutation. High-throughput SNP genotyping was performed using Taqman assay on the ABI prism 7900HT. A biallelic CA-repeat located at the 3 end of SOD1 was genotyped using SSCP on A4V, Caucasian controls and 55 Chinese control samples. Haplotype frequencies and association statistics were estimated using Haploview. P-values less than 0.05 were considered statistically significant. We used a Bayesian method for multipoint linkage disequilibrium mapping incorporated in the program DMLE+ to estimate the age of A4V. Several SNPs in a 5.86cM associated with A4V, most notably the CA-repeat (P=1.72e-14) and rs7276171 (3cM from SOD1) (P=1.78e-6). There was no association with non-A4V SOD1 except around SOD1, and none with SALS. This indicated that the association signal spread over 5.86 cM for A4V was due to LD. A single haplotype of 14 SNPs across this 5.86cM region, absent in the controls, associated with A4V (P=1.5e-11) indicating a founder effect. Haplotype of Swedish samples was different from the associated haplotype suggesting that A4V originated on a different DNA fragment in Europe. The allele frequencies of the CA repeat in Chinese were similar to A4V cases rather than Caucasian controls, indicating that the origin of the A4V in North America was possibly in an Asian population. Using haplotype frequency data the estimated age of A4V is 35.1 to 63.1 generations (~880 to 1580 years) with 90 - 99% posterior probability.

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Mutagenic motifs, recurrent mutation, infinite sites, and the sampling of SNP's in human association studies. *T.J. Maxwell[1], K.E. Hyma[1], L.C. Shimmin[2], E. Boerwinkle[2], J.E. Hixson[2], A.R. Templeton[1], MICORTEX.* 1) Dept Biol, Washington Univ, St Louis, MO; 2) Human Genetics Center, UT Houston Health Science Center, Houston, TX.

Under the infinite sites model multiple mutations at a single nucleotide site are not allowed. This model is assumed both explicitly and implicitly in most human population genetic and association studies. Recurrent mutations and site-rate heterogeneity violate this assumption and can have significant impacts on association studies and the estimation of population genetic parameters (Templeton *et al.* 2005, 2000a, 2000b, Yang 1996, Tajima 1996). Templeton *et al.* (2000a) characterized polymorphisms in a 10-kilobase region of the Lipoprotein Lipase gene and found a disproportionate number of observed mutations occurring at biochemically mutagenic motifs. A substantial number of the observed polymorphisms in the region had experienced recurrent mutation. A significant bias of recurrence was found at mutagenic motifs (Templeton *et al.* 2000b). We extend this study to multiple regions in chromosome 19 that have been sequenced for haploid chromosome lines from 60 individuals. A bias towards polymorphisms at these particular motifs was found but varied from region to region. The amount of recurrent mutation is characterized and related to the mutagenic motifs. Some of these mutagenic motifs result in an asymmetric mutational bias that may exacerbate the impact of violating the infinite sites model. A biased sampling scheme of picking older and more frequent polymorphisms is commonly used in association studies and will likely increase the probability of picking SNPs with recurrent mutational histories. This study suggests site-rate heterogeneity is common and recurrent mutation is not rare.

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Genetic variations of non-synonymous mutations in *MBL2* in different human populations provide evidence for recent natural selection. *T. Bernig[1], E. Tarazona-Santos[1], W.S. Modi[2], M. Yeager[2], R.A. Welch[2], S. Chanock[1].* 1) Section on Genomic Variation, POB, National Cancer Institute, Bethesda, MD; 2) Core Genotyping Facility, ATC, National Cancer Institute, Gaithersburg, MD.

Mannose-binding protein (MBL) is encoded by the *MBL2* gene and is a critical component of the innate immunity. Common *MBL2* haplotypes, which are based on 3 linked 5 variants and 3 non-synonymous SNPs in exon 1 (known as B, C and D), influence serum level and functional activity of MBL. Previously, we have described the nucleotide diversity and haplotype pattern across the entire *MBL2* gene in 102 individuals from the SNP500Cancer initiative. Now we have selected 15 tagging SNPs, which include the 6 linked variants that compromise the secretor haplotypes. We studied these variants in the CEPH Human Genome Diversity panel (1056 individuals from 52 populations) to describe the geographical pattern of genetic variation in *MBL2*. We performed the analysis with all 15 SNPs as well as with the 6 functional SNPs separately with the assumption that natural selection has played a more significant role in shaping the patterns observed on the latter group. For the 15 SNPs, our analyses show a low level of differentiation among populations (F_{ST} 0.057, $p<0.001$) and among geographic groupings of populations (Africans, Europeans, Middle East, Central/South Asians, East Asians, Oceanians, Native Americans, F_{CT} 0.041, $p<0.001$). The within-population diversity significantly decreases with an increasing distance from East Africa (Spearman =-0.659, $p<0.0001$). The 6 functional SNPs show evidence of higher levels of differentiation (F_{ST} 0.112 $p<0.001$; F_{CT} 0.093, $p<0.001$). Whereas the C-allele was only seen in populations close to East Africa (< 10,000 km), the within-population variability enlarges for the functional B-allele with distance from East Africa (Spearman =0.373, $p<0.007$). These differences among the pattern of the 15 SNPs and the 6 functional SNPs suggest that natural selection has contributed to the observed high frequencies of variant B or C alleles in populations for which there is selective advantage for heterozygosity. Additionally, we will provide extensive resequencing data to prove this assumption.

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Evidence for natural selection in the HAVCR1 gene: high degree of amino-acid variability in the mucin domain of human HAVCR1 protein. *T. Nakajima[1, 2], S. Wooding[3], Y. Satta[4], N. Jinnai[2], S. Goto[2], I. Hayasaka[5], N. Saitou[6], J. Guan-jun[7], K. Tokunaga[8], L.B. Jorde[3], I. Inoue[2], M. Emi[1].* 1) Inst Gerontology, Nippon Medical Sch, Kawasaki, Japan; 2) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 3) Department of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, UT; 4) Department of Biosystems Science, Graduate University for Advanced Studied, Hayama, Japan; 5) Kumamoto Primates Park, Sanwa Kagaku Kenkyusho Co. Ltd, Kumamoto, Japan; 6) Division of Population Genetics, National Institute of Genetics, Mishima, Japan; 7) Red Cross Blood Center of Harbin, Harbin, China; 8) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

The family of genes encoding T-cell immunoglobulin and mucin-domain containing proteins (Tim), which are cell-surface molecules expressed in CD4+ T helper cells, has important roles in the immune system. Here we report three unusual patterns of genetic variation in the human hepatitis A virus cellular receptor 1 gene (*HAVCR1*) that are similar to patterns observed in major histocompatibility complex (MHC) loci. First, levels of polymorphism in exon 4 of *HAVCR1* were exceptionally high in humans (nucleotide diversity () = 45.45 x 10-4). Second, non-synonymous substitutions and insertion/deletion variants were more frequent than synonymous substitutions in that exon (10 out of 12 variants). The rate of the mean number of nucleotide substitutions at non-synonymous sites to at synonymous sites at *HAVCR1*-exon 4 is >1 (P_A/P_S=1.92 and $_A /_S$ =2.23).'@Third, levels of divergence among human, chimp, and gorilla sequences were unusually high in *HAVCR1* exon 4 sequences. These features suggest that patterns of variation in *HAVCR1*, like those at MHC loci, have been shaped by both positive and balancing natural selection in the course of primate evolution. Evidence that the effects of natural selection are largely restricted to the mucin domain of HAVCR1 suggests that this region may be of particular evolutionary and epidemiological interest.

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Stratified linkage analysis of sarcoidosis based on admixture in a sample of African-American families. *C. Gray-McGuire[1], C.L. Thompson[1], B.A. Rybicki[2], M.C. Iannuzzi[3], R.C. Elston[1], S.K. Iyengar[1].* 1) Dept of Epidemiology and Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Dept of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, MI; 3) Division of Pulmonary, Critical Care and Sleep Medicine, Mount Sinai School of Medicine, New York, NY.

Sarcoidosis is an autoimmune disorder of unknown etiology. In the United States, African-Americans are more frequently and more severely affected than Caucasians, females accounting for a large majority of the cases. The first genome scan that was conducted in African-American families ascertained through two siblings affected with sarcoidosis indicated linkage to chromosome 5 and showed marginal evidence of linkage to 6 other regions (Ianuzzi et al, Genes Immun 2005). Because of the known admixture in African-Americans with Caucasian and other populations, we evaluated these linkages in light of the admixture in our sample. The population structure of our sample was inferred via the program STRUCTURE, using the 380 microsatellite markers from our genome scan. Evidence of two subpopulations was found, with seven of the 229 families estimated to be 98% of one subpopulation, 118 estimated to be 98% of another subpopulation, and four of the families representing a mixture of the two subpopulations. The linkage analysis was repeated on the families in these two respective subpopulations using the genome scan marker set. The stratified analysis suggests that several of the regions identified in the original scan are due to subpopulation one (1p22, 11p15 and 17q21) or subpopulation two (5p15-13 and 20q13), while others remain significant in both (2p25, 5q11, 5q35 and 9q34). Evidence for a new locus only in subpopulation two (2q37) was also found. This analysis demonstrates the utility of admixture information to identify more homogeneous subsets of families in studies of complex disease. It also provides evidence for multiples genes influencing sarcoidosis and that these genes may be population specific. Further analysis to better characterize the subpopulations is underway.

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De novo gene conversion in the RCA cluster (1q32) causes mutations in complement factor H associated with atypical hemolytic uremic syndrome. *J. Goodship[1], L. Strain[1], M. Jackson[1], S. Heinan[2], P. Zipfel[2], P. Sánchez-Corral[3], S. Rodríguez de Córdoba[3].* 1) Inst Human Genetics, Univ Newcastle upon Tyne, Newcastle on Tyne; 2) Hans Knoell Institute, Jena; 3) Departmento de Immunologia, Centro de Investigaciones Biologicas, Madrid.

CFH mutations are associated with atypical hemolytic uremic syndrome (aHUS; OMIM 235400). CFH is in the RCA (Regulators of Complement Activation) gene cluster on human chromosome 1q32, which contains more than 60 genes of which 15 are complement related genes. There is a high degree of sequence identity between the genes for factor H (CFH) and the five factor H-related proteins (CFHL1, CFHL2, CFHL3, CFHL4 and CFHL5). There are two nucleotide differences between the coding region of the final exon of CFH and CFHL1 leading to serine/leucine and valine/alanine differences. We have identified two patients in whom S1191L and V1197A have occurred in combination as de novo events. In both cases the changes occurred on the same allele. The amplicon encompassed three upstream intronic single nucleotide difference between CFH and CFHL1 and four downstream single nucleotide differences in the 3 untranslated region. In each case the changes affected only the two coding nucleotides, compelling evidence for gene conversion. Whilst there is circumstantial evidence for gene conversion in a number of diseases unambiguous evidence from de novo events is rare. In our cohort of patients with aHUS, we have identified CFH mutations in 25 families or individuals. 3 (including the 2 de-novo cases) are S1191L and V1197A together, 4 are S1191L and 2 have V1197A. We have confirmed in functional assays that these changes, alone or in combination, result in impaired protection of host surfaces against complement activation. We conclude that gene conversion events represent 9/25 (36%) of CFH mutations in the HUS patients we have studied.

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Common polymorphic deletions in the human genome. *S.A. McCarroll[1,2], S.B. Gabriel[2], M.J. Daly[1,2], D.M. Altshuler[1,2].* 1) Massachusetts General Hospital, Boston, MA; 2) The Broad Institute, Cambridge, MA.

The specific locations, population distribution, and history of structural variants in the human genome are largely unknown. We would like to identify parts of the genome that are missing in many individuals; to discover and localize such deletions on the scale of specific exons and regulatory elements; and to distinguish between ancestral and recurring structural mutations. With these goals in mind, we have developed an approach for using SNP genotypes to discover, localize, and learn the population genetic history of common polymorphic deletions. Our approach is based on the footprint that segregating deletions leave in SNP genotypes, in the form of physical clusters of apparent Mendel conflict, Hardy-Weinberg disequilibrium, and null genotypes. Applying this approach to HapMap genotypes from 270 individuals from three continental populations, we discovered hundreds of polymorphic deletions ranging in size from 1 to 750 kb, including more than 100 common deletions. These optional components of the human genome include expressed genes thought to contribute to drug response, immune response, olfaction, and sex steroid metabolism. To determine the population genetic history of common deletions, we have typed them in hundreds of individuals from the HapMap populations and integrated this structural variation data with SNP haplotype data from the same individuals. Most common deletions appear to result from ancestral mutations that have been inherited by descent. The overwhelming majority of common deletions are in linkage disequilibrium with nearby SNPs and are therefore detectable in whole-genome disease association studies, particularly if such studies are designed with knowledge of a whole-genome deletion map.

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Methods for dating of origin of mutations by using diversity at microsatellites linked with the site of the mutation. *X. Sheng, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Linkage studies or positional cloning analyses have identified disease-causing mutations for a number of genetic disorders and clinical phenotypes. Such data often use the presence of microsatellite markers at close vicinity of the mutation-site. The AIDS-resistance CCR5-delta32 mutation is one such example, for which the proportion of mutation-carrying chromosomes that preserved the same microsatellite haplotype has been used to date the time of occurrence of the CCR5 mutation as 27.5 generations, or approximately 690 years. Coalescence theory provided this estimate of the time of occurrence of the mutation, assuming that the mutation rate (0.001/locus/generation) at the microsatellite locus and the recombination rate (0.005 per meiosis) between the microsatellite locus and the site of mutation are both known. Since microsatellite mutation rates have a large inter-locus variance, we provide an alternative method of dating the origin of the mutation, in which haplotype retention proportion as well as allele size variance at the microsatellite locus on chromosomes carrying the mutation are jointly used to estimate the time of origin and the mutation rate at the microsatellite locus simultaneously. Recombination rate, in this procedure, is estimated from the physical map distance of the site of mutation and the microsatellite locus. Our numerical computations which include information on the dinucleotide marker more distant from the CCR5-delta32 mutation site suggest that the date estimate (22.7 generations) with the conjoint use of the allele size variance data agrees well with the estimate (19.2 generations) based on haplotype retention probability alone and the estimated mutation rate ($7.87*10^{-5}$) is more consistent with dinucleotide mutation rates published in the literature. Coalescence-based simulation experiments also provide the relative efficiency of the time estimator based on haplotype retention and allele size variance in comparison with the one based on the haplotype retention probability alone. (Research supported by the NIH grant GM 41399 to RC).

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Relationship of Ethnic Groups in Western China --Insights from Patrilineal Lineage Genetics. *Y. Chen, J. Chu.*
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There are 56 different ethnic groups residing in China, and the research on genetic structure will help us to understand the genetic polymorphism and the relationship of these Chinese ethnic groups. To investigate the relationship between the ethnic groups in western China from the point view of patrilineal lineage genetics, the genetic polymorphism of the YAP+ and Y-SNPs haplotype were studied. For the genetic polymorphism of the YAP+, we selected the DNAs of 1006 healthy individuals in 27 ethnic groups in China. The result showed that the YAP+ frequencies in the following populations were: Zang 36.7%,Tuzu 23.8%,Yi 18.4%,Pumi 11.3%,Tajik7.4%,Bai.7%,Jino 5.1%,Shandong Han 4%,Mulao 2.7%,Maonan 2.2%. The YAP+ element could not be detected in Gansu Han, Yunnan Han, Zhuangzu, Dai, Li, Nu, Lisu, Naxi, Lahu, Dulong, Hani, She, Weiwuer, Sala, Kerkizi, Dongxiang and Wa. In these ethnic groups, the genetic studies were reported for the 1st time to conduct on Tajik, Mulao, Maonan, Sala, Kerkezi, Dongxiang and Jino populations. Conclusion: The Sino-Tibetan language families (Zang, Tu, Yi, Pumi, Jino, and Bai) carried high YAP+ frequency. Sala, Tu and Tajik known to have been originated from Central Asia by history and linguistics, also had high YAP+ frequency. Mulao and Maonan, presumably originated from Baiyue ancient ethnic groups, had comparatively high YAP+ frequency. We found that YAP+(H2 and H3) were also carried by Baiyue groups, which is different from other previous reports: ethnic groups originated from Central Asia carries a very low YAP+ frequency. Besides, 6 ethnic groups patrilineal genetic structure data of 13 Y-SNPs haplotype were analyzed, which were Tajik,Dongxiang,Weiwuer,Kerkezi,Mulao and Maonan. The results showed that Mulao and Maonan carried typical Baiyue genetic characteristics. Three ethnic groups (Kerkezi, Dongxiang and Weiwuer) of the Altai Language family and one ethnic group of Indio-European Language (Tajik) also carry similar Y-SNPs haplotype distribution. The genetic characteristics of these ethnic groups are similar to their linguistics,histories and archeology relationships .

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Haplotypes and LD of the DTNBP1 locus in worldwide populations. *K.K. Kidd, A.J. Pakstis, W.C. Speed, J.R. Kidd.*
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DTNBP1 on 6p22.3 has been implicated as a locus with alleles conferring susceptibility to schizophrenia in studies of several European populations. As background for planning further studies we have typed 16 SNPs across ~180 kb encompassing the gene. A 112 kb segment toward the 3' end of the gene shows strong LD in most of the 42 population samples studied but with one 11-site haplotype at a frequency of at least 50% in most populations. Within this segment the pattern of sub-segments with stronger LD is remarkably consistent among populations including African populations. However, the sub-segments with stronger LD do extend farther in the East Asian and Native American populations. Two 11-site haplotypes that exist in moderate frequencies in African and in European populations are generally absent in the East Asian and Native American samples. One 11-site haplotype that occurs at 15 to 20% in the two African Pygmy populations is rare in other African populations and is not seen outside of Africa. LD does not extend to either end of the gene except in a few of the smaller, more isolated populations. When all 16 sites are considered, 32 haplotypes occur among all populations at common frequencies of 5% or more with any single sample having 3 to 9 of these; no haplotype occurs at more than 20% frequency in any African or European population and most are 10%. There is greatly reduced variation in East Asian and Native American populations with only three haplotypes accounting for 75% of chromosomes in most East Asian populations and two of these accounting for 50% of chromosomes in Native American populations. The absence of LD between variation at the 5 end of the gene and the common haplotypes through much of the coding region may explain the association of schizophrenia with different haplotypes in different populations if regulatory variation is what is etiologically relevant. Supported in part by NIH GM57672 and MH041953.

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Analysis of ancient mitochondrial DNA lineages in Yakutia. *S.A. Fedorova[1], T. Ozawa[2], A.D. Stepanov[3], E.K. Jirkov[3], L.L. Alekseeva[3], M. Adoyan[4], J. Parik[4], R. Villems[4].* 1) Department of molecular genetics, YaNC of RAMS, Yakutsk, Republic Sakha (Yakutia), Russian Federation; 2) Laboratory of Geobiology of Nagoya University, Nagoya, Japan; 3) Institute of Archaeology and Ethnography, Yakutsk, Russia; 4) Estonian Biocenter and Tartu University, Tartu, Estonia.

Molecular genetic analysis of ancient human remains are mostly based on mitochondrial DNA due to its better preservation in human skeletons in comparison with nuclear DNA. We investigated mtDNA extracted from human skeletons found in graves in Yakutia to determine their haplotypes and to compare them with lineages of modern populations. Ancient DNA was extracted from fragments of three skeletons of Yakut graves at At-Dabaan, Ojuluun and Djaraama sites (dating XVIII century) and two skeletons of Neolithic graves at Kerdugen site found in central Yakutia. Three fragments of HVSI of mtDNA were amplified 15986-16149, 16106-16271, 16209-16401. After the PCR the products were purified and the fragments of HVSI were cloned in plasmide M13. 5 - 10 clones were analyzed for each DNA fragment. Sequence reactions were performed using DYEnamic ET terminator cycle sequencing kit, Amersham Pharmacia Biotech. Haplogroups were identified by mutations against the Cambridge reference sequence. Five different haplotypes belonging to specific Asian haplogroups were identified. Lineages of mtDNA of Yakut graves belong to haplogroups C4a, D5a and B. This haplotypes were revealed earlier in the mitochondrial gene pool of modern Yakut population (Fedorova, 2003; Puzirev, 2003, our unpublished data). Our results indicate the continuity of mitochondrial lineages in the Yakut gene pool during the last 300 years. Haplotypes of two humans from Kerdugen site graves belong to haplogroups A and G2a/D. We compared these haplotypes with that of 23048 Eurasian individuals, 860 of them from Yakutia. No exact matches were found in modern populations of Yukaghirs, Chukchi, Eskimos, Koryaks and Itelmens. Phylogenetically close haplotypes (1 mutation) were found in populations of Evens, Evenks and Yakuts, as well as in some populations of Central Asia, South Siberia, Mongolia and North China.

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Princess Ngonnso' and the foundation of the royal house of Nso'. *K.R. Veeramah[1], D. Zeitlyn[1], V.G. Fanso[2], B.A. Connell[3], M.E. Weale[1], N. Bradman[1], M.G. Thomas[1].* 1) The Centre for Genetic Anthropology, University College London; 2) University of Yaoundé, Cameroon; 3) York University, Toronto.

Background: The Nso' (population >300,000) live in the region of their pre-colonial empire in the western Grassfields of Cameroon. They are divided into four caste-like groups (CLGs), including a royal CLG, the *wonto'*. Accounts of the origin of the Nso' are recorded in multiple oral traditions of which the most common tells of a Princess Ngonnso' migrating from the Tikar region to the east and encountering a group of indigenous hunter-gathers (the Visale) amongst whom she settled some 250-700 years ago. Opinion differs as to whether the Princess came with a Tikar husband or married a Visale chief but it is agreed that her son became the first Fon (king) of Nso' from whom all subsequent Fons claim descent along the paternal line. **Aim:** We investigated which of the different variants (if any) of the oral history are consistent with genetic data obtained by analysing the non-recombining portion of the Y-chromosome (NRY) and mitochondrial DNA (mtDNA). We formulated prior hypotheses of the distribution of NRY and mtDNA types consistent with (1) alternative versions of the oral history and (2) none of the alternatives. The NRY of males from the Nso' and 12 other Grassfields and Tikar region groups were typed and the mtDNA HVS-1 and key coding region markers characterised in the Nso'. **Results:** We identified an NRY type characteristic of hunter-gatherer populations, thought to have been common before the expansion of the Bantu-speaking peoples, at a high frequency in the *wonto'* (>50%) but at much lower frequencies in the other CLGs and at only 0.001% in the non-Nso' groups. In contrast, we found no significant difference in the frequencies of mtDNA types among the Nso' CLGs (P>0.285)). These results are consistent with the most common origin story of the Nso': that the father of the first Fon was more likely to have been a Visale rather than a Tikar. This study demonstrates the usefulness of genetics, complementing other disciplines such as anthropology, linguistics and archaeology, in recovering the histories of peoples of sub-Saharan Africa.

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Admixture Linkage Disequilibrium in African American. *S. Xu[1], H. Wang[2], Y. Wang[2], Y. Liu[2], Y. Wang[2], X. Chu[2], Y. He[1], Y. Wang[1], W. Huang[2], L. Jin[1,2,3].* 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) Center for Genome Information, University of Cincinnati, Shanghai, USA.

Linkage disequilibrium due to admixture (ALD) has important value in locating genes underlying diseases or phenotypes of distinctive ancestral history. In this study, we examined ALD in a sample of 48 African-Americans (AfAs) of 24,339 SNPs encompassing the entire chromosome 21. Overall 1,740 ancestry informative markers were selected for more detailed analyses. Putative ancestral origin of chromosomal segments in individual AfAs was inferred using 60 CEPH parents and 60 Yoruba parents in the international HapMap Project. The average European ancestry proportion of AfA individuals was estimated as 21.1% ranging from 0.3% to 78.6%. The average length of chromosomal blocks of African origin is 7 cM, while that of European origin is 2 cM. Interestingly, ALD in our sample extends on chromosome 21 much shorter than those of other studies involving other chromosomes and different African Americans. The level and pattern of admixture linkage disequilibrium is likely to specific to certain genomic regions and populations that have different admixture levels. The current results should have great implication for admixture mapping.

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The genetic structure of human populations studied through short insertion-deletion polymorphisms. *L. Bastos-Rodrigues[1], J.R. Pimenta[2], S.D.J. Pena[1,2].* 1) Bioquímica e Imunologia, UFMG, Belo Horizonte, MG, Brazil; 2) GENE-Núcleo de Genética Médica, Belo Horizonte, MG, Brasil.

In 2002 Rosenberg et al. published a landmark study of the structure of human genome diversity without prior population definition, by using a set of 377 microsatellites and the HGDP-CEPH Human Genome Diversity Panel. They reported that the populations included in the study were best structured into genetic groupings that tightly corresponded to five regions: America, Sub-Saharan Africa, East Asia, Oceania and a cluster composed of Europe, Middle East and Central Asia. They also observed that the within-population differences among the individuals accounted for 93-95% and that the among-regions variation was only 3.6% of the total genetic variance. We have also studied the HGDP-CEPH Diversity Panel (1064 individuals from 52 populations) with a set of 40 biallelic slow-evolving short insertion-deletion polymorphisms (indels). We loci were widely spread in the human chromosomes and in their vast presented allelic frequencies in the range 0.4-0.5 in European population. We confirmed the partition of worldwide diversity into five genetic clusters that correspond to major geographic regions. Analysis of molecular variance (AMOVA) demonstrated that, within populations, the differences among individuals accounted for approximately 86% of genetic variation, while those differences among geographic regions constitute approximately 12%. With the indels, we have also disclosed an among-regions component of genetic variance considerably larger (12.1%) than had been estimated using microsatellites. Our results agree much better with estimates obtained in several previous studies and indicate that the probable reason for the underestimation of this component of variation in the analysis of Rosenberg et al. (2002) was not the sampling scheme, as they posited, but rather their use of fast evolving microsatellite markers. Our study demonstrates that a set of 40 well-chosen biallelic markers is sufficient for a detailed characterization of human population structure.

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Assessment of global genetic diversity of the Azorean population by 24 microsatellite loci. *C.C. Branco[1,2], P.R. Pacheco[1,2], R. Cabral[1,2], L. de Fez[1,2], B.R. Peixoto[1,2], L. Mota-Vieira[1,2].* 1) Molec Gen & Pathology Unit, Hosp Divino Espirito Santo, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The pattern of genetic diversity is the result of many evolutionary processes and, its knowledge is crucial to understand how history and demography shaped the structure of a population. Here, we describe the genetic diversity of the Azorean population, and compare it with a sample from mainland Portugal. We studied 24 microssatelite loci (TPOX, D3S1358, FGA, CSF1PO, D5S818, D6S265, TNF, D7S820, D8S1179, D9S762, D10S525, TH01, VWA, D13S317, D14S306, FES/FPS, D16S539, D17S976, D18S51, D19S433, D20S161, D21S11, D22S417 and DXS983) in 222 Azorean and 31 mainland Portuguese unrelated blood donors. These short tandem repeat (STR) markers were typed by Polymerase Chain Reaction (PCR) with fluorescently labelled primers. Allelic frequencies were statistically analysed using Arlequin v.2.0. Comparisons between samples were performed by Genepop web version and GDA. Phylogenetic trees based on Neis genetic distance were constructed with DISPAN and PHYLYP softwares. Analysis of microsatellite loci shows that the Azorean population presents lower average gene diversity (0.776) when compared with mainland Portugal (0.781). Moreover, we observe a higher inbreeding (FIS=0.0531) in the Azoreans. In both samples, the markers TPOX and D17S976 showed the lowest (0.6) and highest (0.9) values of gene diversity, respectively. The admixture coefficient (mY) reveals Portuguese as the major contributors to the genetic background of the Azoreans. This observation is corroborated by the dendrogram, in which Azores clusters with Portuguese, Spanish, Italians, and Belgians, apart from Moroccans and Cabo Verdeans. Taken together, these data complement settlement history of the Azorean population and will be important for future studies of genetic diseases. (claudiacbranco@hdes.pt). Funded by DRCT (Azores).

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How likely is this structural change to be deleterious? *K. Li, L.L. Chen, H.L. Gao, B. Scaringe, S.S. Sommer.*
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When an amino acid substitution not known to be a polymorphism/rare variant is found in a patient on DNA testing, what is the likelihood that it is deleterious? A "likelihood algorithm" is being validated and optimized for the factor IX gene, a uniquely advantageous system in which more than 1000 unique substitutions cause hemophilia B and more than 50 wildtype sequences across species demonstrate more than 1000 neutral amino acid substitutions. The algorithm assigns "deleterious probabilities" with confidence intervals to any given amino acid substitutions. The algorithm can be adapted to other genes by inputting the wildtype sequences known, and the deleterious and neutral variants available from mutation databases and other sources. Further validation of the likelihood algorithm with other genes will be required to demonstrate it's general utility. A likelihood algorithm may provide clinicians with an objective, real time estimate of the likelihood that a functionally deleterious missense mutation has been defined.

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No association was found between three SNPs in the ghrelin precursor gene with variations in body fat and serum lipid parameters in the Newfoundland population. *G. Sun, G. Martin.* Dept Genetics, Memorial Univ Newfoundland, St John's, NF, Canada.

Ghrelin has been recognized for its involvement in food intake and control of energy homeostasis. However, the roles of genetic variations in the ghrelin precursor gene (GHRL) on body compositions and serum lipids are not clear in humans. Our study was designed to investigate three single nucleotide polymorphisms (SNPs) within GHRL to determine their relationship with body fat percentage (%BF), trunk fat percentage (%TF), and serum lipids in a large study (1182 subjects) from the province of Newfoundland and Labrador (NL), Canada. %BF and %TF were measured using dual-energy X-ray absorptiometry. Serum concentrations of glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and risk ratio were determined. Genomic DNA was extracted from whole blood and all subjects were genotyped for an A/G polymorphism (mis-sense mutation) found in exon 1 (rs35684), a C/T intronic polymorphism located between exon 2 and exon 3 (rs2075356), and an A/G intergenic polymorphism found near the 3 untranslated region (rs26311) of GHRL using TaqMan Validated SNP Genotyping Assays and an ABI 7000 SDS. The frequencies for alleles A and G of the SNP located in exon 1 were 0.73 and 0.27, frequencies for alleles C and T of the intronic SNP were 0.11 and 0.89, and frequencies for alleles A and G of the SNP located in the 3 intergenic region were 0.88 and 0.12, respectively. All SNPs investigated were in Hardy-Weinberg equilibrium according to Chi-Square analysis. There was no evidence of an allele, genotype, or haplotype association between any of the variant sites with body compositions or serum lipids in our study. Analyses were also repeated after adjusting for possible confounding factors: age and sex for body compositions and age, sex, and %BF for serum lipids. The results, however, remained consistent. The results of our study do not suggest a significant role for the genetic variations in the GHRL gene in the differences of body fat and serum lipid profiles in the NL population.

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Extensive polymorphism of A^1, A^2, B, O^1, O^2 alleles and identification of five novel alleles at the ABO blood group locus in a Kuwaiti population. *M.M. El-Zawahri[1], Y.A. Luqmani[2], S.P. Yip[3], P.S. Choi[3].* 1) Dept Biological Sci, Fac Sci, Kuwait Univ, Kuwait; 2) Dept Pharmaceutical Chem, Fac Pharmacy, Kuwait Univ, Kuwait; 3) Biomedical Sci, Hong Kong Polytechnic Univ, Hong Kong.

The genetic polymorphism described in the present study is the first demonstration of an extensive variability linked to alleles and sub-alleles of the ABO blood group gene in the Kuwaiti population. Kuwaiti population is composed of multiplex Kuwaiti families, mainly from 3 major Asian ancestral populations from Saudi Arabia, Iraq and Iran. Genomic DNA samples from 521 unrelated Kuwaiti donors were analyzed for the ABO alleles and sub-alleles. Genotyping of 355 DNA samples was done by a multiplex PCR-FRLP analysis. Fragments of 252(251 for O^1) bp and 843(842 for A^2) bp cover the 2 major exons 6 and 7 of the ABO gene were amplified and digested with HapaII and KpnI. Thirteen genotypes were found from the A^1, A^2, B, O^2, and O^2 alleles in the Kuwaiti sample population analyzed. Two genotypes (A^2A^2 and O^2O^2) were not detected. The frequencies of the five alleles are 0.1579, 0.0199, 0.1566, 0.6243 and 0.0413, respectively. 166 DNA samples were genotyped by a multiplex PCR-SSCP analysis. Three PCR fragments were amplified from exon 6, the 5 end and the 3 end of exon 7. Genotypes of classical alleles (A101, A102, A201, B101, O101, O201 and O303) could easily be identified. Non-standard SSCP patterns were investigated by DNA sequencing of exons 6 and 7 of the ABO gene, and intron 6 if necessary. Sixteen alleles producing a total of 31 genotypes were identified among the samples analyzed. In addition to the six classical alleles, ten variant alleles were found: one A, one B and 8 O (total frequency = 0.1175). Five of these 10 variant alleles are novel alleles defined by SNPs in exon 7 (527GA, 649CT, 687CT, 689GA and 1116GA). Three new base substitutions result in amino acid changes. The present data provide an exciting new dimension to our understanding of human genetic polymorphism in the Kuwaiti population. Supported by Kuwait University Research Administration Grant SZ 01/00 and School of Nursing, The Hong Kong Polytechnic University.

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Geographic Region-specific selection in *ADH* Class I genes. *Y. Han[1], H. Oota[1], S. Gu[1], M. Osier[2], A. Pakstis[1], J. Kidd[1], K. Kidd[1].* 1) Department of Genetics, Yale University School of Medicine, New Haven, CT; 2) College of Sciences, Biological Sciences, Rochester Institute of Technology, Rochester, NY.

ADHs are a family of enzymes that catalyze the reversible oxidation of alcohol to acetaldehyde. Seven human *ADH* genes have been identified. They exist in a cluster extending > 360kb on the long arm of chromosome 4 (4q21). We have typed 42 human worldwide populations for 36 SNPs across the three Class I *ADH* genes. These genes share 95% sequence identity as a result of duplication at the base of the primate lineage and selection to maintain function. The middle gene, *ADH1B*, has a functional variant associated with protection against alcoholism (*ADH1B*47His*) in exon 3 at > 60% in East Asian populations believed to be so frequent because of region-specific positive selection. The F_{st} for this variant, 0.48, is among the highest seen for any of the *ADH* sites in these populations. Markers on either side, from 10kb upstream to 40kb downstream, also show high F_{st} values. Half of the sites (8 out of 16) have F_{st} values above the 95^{th} percentile of the reference F_{st} distribution for these 42 populations; these elevated values do not extend to the next gene upstream, *ADH7*, or the next gene downstream, *ADH6*. In both cases the F_{st} values are approximately the mean for the reference F_{st} distribution, i.e., about 0.14. Besides F_{st} statistic, another genomic test, Long Range Haplotype (LRH) test also gives statistical evidence for the positive selection for this functional variant in East Asian populations. In most populations, LD does not extend much beyond the individual *ADH* Class I genes. However, we found the extended haplotype homozygosity (EHH) of the most exclusively high frequent haplotype including that functional variant decayed more slowly than those of other haplotypes in East Asian populations. The strong extended haplotype homozygosity (EHH) is found across 10~28kb upstream and 50kb downstream of the *ADH1B* Arg47His variant in East Asian populations. Therefore, both F_{st} Statistics and LRH test support the conclusion that selection has acted around the *ADH1B* functional variant in East Asian populations. [Supported in part by NIH grant AA09379].

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Using Hispanic genetic diversity to infer the haplotype structure of Native Americans. *E. Tarazona-Santos[1], M. Yeager[2], B. Staats[2], R. Welch[2], L. Burdette[2], B. Packer[2], S. Chanock[1].* 1) Section of Genomic Variation, Pediatric Oncology Branch, NCI/NIH, Bethesda, MD; 2) Intramural Research Support Program, SAIC-Frederick and Core Genotyping Facility, NCI-NIH, Bethesda, MD, USA.

Cultural and logistic factors have prevented studies about genomic diversity in Native American. Fortunately, information about their patterns of haplotype structure could be recovered studying admixed populations, whose gene pool has received contributions from Europeans, Africans and Native Americans. The SNP500Cancer initiative (http://snp500cancer.nci.nih.gov/) has generated data about the haplotype structure for more than 500 genes important in cancer in Hispanic, European and African populations. For admixed populations, such as Hispanics, the linkage model of the program Structure uses mapping information from markers and allows estimation of admixture for specific genomic regions on the basis of the admixture linkage disequilibrium, provided that enough marker density is attained. Using this approach and data from more than 5000 SNPs organized in haplotypes and 377 STR, we estimated the admixture contribution of Native American, European and African populations for specific genomic regions. The average admixture across the genome was 17% for Native American, 13% for African and 70% for European, but these estimates vary across the genome. From region-specific admixture estimators and observed haplotype frequencies in Hispanics, European and Africans, we have inferred the haplotype frequencies for an average Native American population for more than 300 genes and tested our inferences against observed frequencies for a subset of genes in a Native American sample (50 Pima-Maya individuals). This approach is particularly useful to identify genes for which haplotype frequencies differ markedly in Native American respect to the other parental populations. It should facilitate estimation of haplotype structure of Native American populations in regions where study is difficult but admixture common. An interesting anthropological application of this approach is the study of genetic diversity in extinct populations that have left traces of their gene pool in current populations.

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STR's, DYS199 and DYS287 markers in three amerindian groups from Venezuelan western. *l. Borjas[1], T. Pardo[2], W. zabala[1], M.A. Sanchez[1], M.G. Portillo[1], J.A. Aranguren[3].* 1) genetic, university of zulia, Maracaibo, Venezuela; 2) biological research institute, University of Zulia; 3) genetic department, faculty of veterinary, University of Zulia, Maracaibo, Venezuela.

Zulia state is located at northwest of Venezuela, has between its main indigenous groups, Wayúu, Bari and Yukpa, which are derived from three main linguistic branches: Arawaca, Chibcha and the Caribbean respectively. In order to clarify the origin and their structure, we analyzed 78 chromosomes of masculine individuals not related, Wayúu (50), Barí (16) and Yukpa (12). The STRs systems were investigated: DYS19, DYS389I/II, DYS390, DYS391, DYS392 DYS393, DYS437, DYS438, DYS439 and DYS385 a/b. In adition, DYS199 and DYS287 markers (polymorphism of insertion Yap Alu) were characterized. The characterization was made in a ABI 310 and electroforesis in gel of agarosa to 2 % according to the case. The values of genic and haplotypic diversity, AMOVA and the obtaining of a distance matrix were calculated through the Arlequín program, version 2000. The study of Principal Components Analysis was made (PCA) using SAS program. The degree was considered in addition to mixture of parentals populations through the program Admix 3. The results generated were compared with data reported in literature for other amerindian populations. According to the STR's, the groups Barí and Yukpa have 100 % of amerindian component whereas the Wayúu group showed mainly amerindian component followed of the European contribution. Negative Yap was present in all the natives of the three groups. The Bari and Yukpa only showed ancestral T allele, whereas the Wayúu has 40 % of C allele. The three ethnic groups have different genetic structures. These data keep agreement with the historical documentation about the peoplement from the Zulia state. This work was partially subsidized by CONDES, according to Nº CC-457-01.

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High resolution Y-chromosome analysis of surname association with paternal relatedness. *S.R. Woodward[1,2], N.M. Myres[1], J.B. Ekins[1], J.E. Ekins[1], L.A.D. Hutchison[1], L. Layton[1], M.L. Lunt[1], S.S. Masek[1], A.A. Nelson[1], M.E. Nelson[1], K.L. Pennington[1], U.A. Perego[1], J.L. Peterson[1], K.H. Ritchie[1], T. Tolley[1].* 1) Sorenson Molec Genealogy Fndn, Salt Lake City, UT; 2) Dept Molecular Biol, Brigham Young Univ, Provo, UT.

The correspondence of surname and relatedness has been used as an indicator of population subdivision in both epidemiological and population migration studies. Recently, a limited number of studies have used the Y-chromosome to assess the validity of using surname as a measure of paternal relatedness due to its analogous paternal transmission. These studies have reported varied results depending on the particular surname, the resolving power of the marker system, and the population(s) under investigation. To further characterize the correlation of surname and paternal relatedness we have analyzed 9,100 male samples representing 5,556 surnames at 36 Y-chromosome STR loci. We report surname frequencies and classify non-unique surnames as mono or polyphyletic. Paternal genealogies were used to identify potential ancestral and/or geographic origins for each lineage within each of the polyphyletic surname groups. These findings have application to estimating non-paternity rates, compiling forensic databases, population genetic studies, epidemiological studies, and ancestry testing.

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High Throughput Genotyping of Potentially Functional Polymorphisms in Human Immune Response Genes. *H. Yonath[1], K. Bucasas[1], F. Yu[1], M. Jain[2], V. Carlton[2], J. Ireland[2], S. Fernbach[1], G. Silva[3], R. Gibbs[1], J. Belmont[1]*. 1) Baylor Col Medicine, Houston, TX; 2) ParAllele BioScience, South San Francisco, CA; 3) UNICAR Foundation, Guatemala.

Variation in genes that regulate the quality, intensity, and duration of the innate and adaptive immune responses (IR) are known to play important roles in resistance to infection, vaccine response, and susceptibility to autoimmune disorders. We are developing large standard panels of assays for human IR gene variants that may be employed in a variety of disease projects. In Stage I of this program we have designed assays in 286 IR genes, including HLA Class I, II, Toll, Chemokine, Interleukin, and Interferon pathways. 2987 SNPs were selected, according to the following order of preference: non-synonymous, synonymous, 5UTR, 3UTR, and conserved intronic. Molecular Inversion Probes (MIP) for 2529 SNPs passed the design criteria and were synthesized. To validate the assays, we genotyped anonymous control DNA samples from 4 different ethnic groups: 27 samples from African- American, 27 European-American, 25 Hispanic (Mexican American), and 25 American Indigenous (Mayan). The raw assay conversion rate was 87%. For further analysis we imposed the following QC criteria: call rate 90%, 2 duplicate concordance errors, and 1 homozygotes for the more common allele. There were significant differences in the number of non polymorphic markers between the different populations, 268 in the African- American, 394 in the European-American, 348 in the Hispanic, and 661 in the Mayan. Numerous SNPs showed population-specific departures from Hardy Weinberg equilibrium (HWE): 183 in the African- American, 182 in the European-American, 179 in the Hispanic, and 142 in the Mayan. Although IR genes present some unique challenges in assay design, genotyping error is unlikely to explain all of the observed HW disequilibrium. Comparison of these results with Phase I HapMap and formal tests for selection are underway. Selection of putative functional SNP markers represents an alternative to haplotype tagging strategies in that one might detect direct associations with medically important phenotypes.

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Characterization of family-specific LD patterns in Sorenson Molecular Genealogy Foundation dataset. *K.L. Pennington[1], K.H. Ritchie[1], J.E. Ekins[1], J.L. Peterson[1], N.M. Myres[1], J.B. Ekins[1], L.A.D. Hutchison[1], L. Layton[1], M. Lunt[1], S. Masek[1], A.A. Nelson[1], M.E. Nelson[1], U.E. Perego[1], T. Tolley[1], S.R. Woodward[1,2].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; 2) Dept. of Microbiology, Brigham Young University, Provo, UT, USA.

Inference of familial relationships is a useful tool in a variety of contexts, such as disease linkage and association studies, forensic applications, and inference of personal histories. To date, Y-chromosomal and mtDNA testing have been used extensively for these purposes. However, little has been done using autosomal DNA due to the complex inheritance pattern involved. Herein we characterize family-specific LD patterns and haplotype frequencies as a tool for inferring family group assignments through relationship probabilities. Five extended Utah family groups ranging from 28 to 142 samples and one dataset of 1095 randomly selected samples were analyzed for significant linkage disequilibrium within twelve autosomal 3-locus STR haplogoups. Additionally, one locus was chosen from each of these haplogroups and LD calculations were run among these unlinked loci. Each of the families showed significantly more LD both within and between haplogroups than did the random dataset. Haplogroups and locus pairs demonstrating significant LD were further analyzed to find identify haplotypes and allele pairs with 10x expected coocurrances based on random allele frequencies. Several haplotypes were found within each family set with frequencies characteristic of that particular family group relative to the other datasets. These haplotype frequencies may be used to assign probabilities of membership of an unknown individual into these family groups.

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Patterns of linkage disequilibrium between SNPs on a Sardinian population isolate and the selection of markers for association studies. *F.M. De La Vega[1], F. Hyland[1], A. Angius[2,3], I. Persico[2,3], T. Woodage[1], M. Pirastu[2,3].* 1) Applied Biosystems, Foster City, CA, USA; 2) Shardna Life Sciences, Cagliari, Italy; 3) Istituto di Genetica delle Popolazioni, CNR, Alghero, Italy.

The patterns of linkage disequilibrium (LD) determine the feasibility of association studies utilizing single-nucleotide polymorphisms (SNPs) to map genes that underlie complex traits. Interest in utilizing population isolates for LD mapping studies has grown considerably, based on the assumption that recent population bottlenecks would result in more extensive LD. We studied the profile of LD in a Sardinian population isolate in a 8 Mb segment of chromosome 22. 771 SNPs were genotyped using the SNPlex Genotyping System, a multiplexed genotyping platform based on the oligonucleotide-ligation assay. 101 unrelated individuals and 313 members of 15 families from the village of Talana in east Sardinia were genotyped. This data is compared with genotypes on a sample of 45 unrelated Caucasian individuals typed for 566 of the same SNPs using TaqMan SNP Genotyping assays, and with the data obtained by the HapMap Project. Among the common set of 566 SNPs, 6.9% were monomorphic in the isolated and 4.4% in the outbred population; and 6.5% versus 3% in the outbred population had a frequency < 5%. Minor allele frequencies were almost identical at 26-27%. The extent of LD in the two populations, however, is quite different. The decay of pairwise D and r^2 with distance is slower in the isolated as compared with the outbred population: the half-life of the decay curve is larger and the plateau is higher. Common haplotype concordance was remarkably high. We constructed metric LD maps for both populations that when plotted against the physical map show a pattern of plateaus (LD blocks), and steps (recombination hot spans) that is similar between the isolate and the outbred populations. The differences in the length of the LD maps for the region imply that useful LD for mapping may extend up to four times longer on the physical map in the population isolate than in the outbred sample, and suggest a cost-effective strategy for marker selection in isolated populations.

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Large-Scale Recombination Rate Patterns Are Conserved among Human Populations. *D. Serre, R. Nadon, T.J. Hudson.* Genome Quebec Innovation Center, McGill University, Montreal, QC, Canada.

In humans, most recombination events occur in a small fraction of the genome. These hotspots of recombination show considerable variation in intensity and/or location across species and, potentially, across human populations. On a larger scale, the patterns of recombination rates have been mostly investigated in individuals of European ancestry and it remains unknown whether the results obtained can be directly applied to other human populations. Here, we investigate this question using genome-wide polymorphism data from three populations produced by Perlegen Sciences Inc. We show that population recombination rates recapitulate a large part of the genetic map information, regardless of the population considered. We also show that the ratio of the population recombination rate estimate of two populations is overall constant along the chromosomes. These two observations support the hypothesis that large-scale recombination patterns are conserved across human populations. Local deviations from the overall pattern of conservation of the recombination rates can be used to select candidate regions with large polymorphic inversions or under local selection.

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Using frequency matched linkage disequilibrium reveals evidence for natural selection and improves SNP marker optimization. *M.A. Eberle, M.J. Rieder, D.A. Nickerson.* Department of Genome Sciences, University of Washington, Seattle, WA.

Linkage disequilibrium (LD) describes the non-random association between single nucleotide polymorphism (SNP) pairs and can be used as a metric when designing maximally informative panels of SNP markers for association studies in human populations. We analyzed the extent of perfect linkage disequilibrium ($r^2 = 1.0$) across the human genome using ~1.58 million SNPs genotyped by Perlegen in three diverse populations of European, Asian and African descent. We show that by matching pairwise SNP combinations based on allelic frequency, LD (r^2) values range from 0 to 1, whereas r^2 between SNPs is always limited to values much less than 1 ($LD_{max} \sim 0.6$) when averaging across the entire frequency distribution. Therefore, frequency matching SNP pairs for the calculation of r^2 provides a more sensitive measure for assessing the decay of LD. Using this LD metric, we demonstrate an increased extent of LD, and a significantly higher fraction of perfectly correlated SNPs in genic regions compared to intergenic regions at distances ranging from 20 kb to more than 400 kb throughout the human genome. In addition, these results were not caused by the effects of increased recombination rates because, compared to intergenic regions, perfectly correlated SNPs in genic regions were more likely to occur in regions of increased recombination. SNP pairs exhibiting perfect LD also showed a significant bias for derived, non-ancestral alleles, which may be indicative of positive natural selection. In addition, we estimated the effect of LD in designing optimal SNP sets for whole genome association studies. We demonstrate that SNP marker selection based on allele frequency matching leads to a dramatic reduction (3 - 10 fold) in the number of SNPs needed for association mapping and could require as few as 100,000 SNPs genome-wide.

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Identification of TUB as a novel gene associated with body weight regulation in man. *M. Hofker[1], R. Shiri-Sverdlov[1], A. Custers[1], J.V. van Vliet-Ostaptchouk[1], P.J.J. van Gorp[1], P.J. Lindsey[2], J. van Tilburg[3], A. Zhernakova[3], B.P.C. Koeleman[3], E.J.M Feskens[4], C. Wijmenga[3].* 1) Dept Molecular Genetics, Cardiovascular Inst, Maastricht, Netherlands; 2) Dept Population Genetics NUTRIM, Maastricht, Netherlands; 3) Division of Medical Genetics, University Medical Center Utrecht, Netherlands; 4) Center for Nutrition and Health, RIVM, Bilthoven, Netherlands.

Previously, we identified a genomic locus on 11p harboring quantitative trait loci influencing obesity in families with type II diabetes (T2D) from the Breda cohort. In the current study, we selected TUB as a functional candidate gene from this locus and performed association studies to determine whether this gene has an effect on T2D and /or obesity. TUB is the founding-member of the tubby-like proteins and is conserved among vertebrate genomes. The tubby mouse which arises from a loss-of-function mutation, presents a syndrome that is characterized by late onset obesity. In a case-control design 443 T2D patients from the Breda cohort and 356 controls were genotyped for 13 single nucleotide polymorphisms (SNPs) along this gene. Although no significant association to T2D was identified, linear regression analysis revealed that a combination of 3 SNPs (SNPs 8, 10 and 2) has a marked effect on BMI (+0.9, +1.8, +1. 2 kg/m2 respectively, p=0.007). We replicated this study in an independent cohort, using 664 individuals that were not selected for T2D. This independent cohort confirmed our initial findings as a significant effect on BMI was found for SNP 2 (+1.5 kg/m2, p=0.008). Combined analysis of both cohorts revealed that the effect of this allele on BMI was +1.2 kg/m2, p=0.004. Importantly, this allele was found to be more common in obese subjects (OR=1.32, p<0.02). The associations observed for SNPs in this gene indicate clearly the influence of TUB on body weight, independent of T2D. Moreover, the minor allele of SNP 2, which has a frequency of 0.28, was found to be more common among obese patients within both study populations, indicating the contribution of this gene to obesity.

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Human mitochondrial SNP genotyping in large cohorts of European ancestry. *B.J. Klanderman[1,2], S. Mazza[1], B.A. Raby[1,2,3], S.T. Weiss[1,2].* 1) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Division of Pulmonary and Critical Care Medicine, Beth Israel Deaconess Medical Center, Boston, MA.

It is well established that particular rare mutations in the mitochondrial genome can lead to severe maternally-inherited disorders; however, there is growing interest in exploring the mitochondrial genome for its contribution to common, complex diseases. To this end, we are developing a panel of mitochondrial single nucleotide polymorphisms (mtSNP) for haplotype tagging of the mitochondrial genome, with the goal of establishing a reliable set of mtSNP TaqMan Genotyping Assays for complete characterization of all common European haplotypes (frequency at least 1%). To construct this set of SNP assays, we are genotyping SNPs from the MITOMAP human mitochondrial genome database (www.mitomap.org). Primer and probe sequences are aligned against mitochondrial and autosomal genomic sequence to ensure their unique binding to one mitochondrial locus, without overlapping other known mtSNPs. All SNPs are genotyped in three large cohorts of self-reported European ancestry: 1030 adult women participating in the Nurses' Health Study, 432 men participating in the Normative Aging Study, and 948 individuals of European descent collected from eight North American cities. To assess population specificity, these SNPs are also typed in 134 African Americans and 128 Mexican Americans. Haplotype tagging SNPs are selected using the BEST algorithm (Sebastiani et al. 2003), and common haplogroup distributions are compared to those previously described by Torroni et al. (1996). These assays will be available as TaqMan Genotyping Assays from Applied Biosystems, Inc., and we anticipate that this set of mitochondrial haplotype-tagging polymorphisms will serve as a useful tool for genetic association studies of common traits.

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An Oceanian lineage of mtDNA is associated with the lower BMI in Solomon men. *R. Kimura[1], J. Ohashi[1], I. Naka[1], T. Furusawa[2], T. Yamauchi[2], K. Natsuhara[2], M. Nakazawa[3], T. Ishida[4], R. Ohtsuka[2], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; 2) Department of Human Ecology, Graduate School of Medicine, The University of Tokyo; 3) Socio-Environmental Health Sciences, Graduate School of Medicine, Gunma University; 4) Department of Biological Sciences, Graduate School of Science, The University of Tokyo.

It is recently suggested that regional variation in human mtDNA was shaped not only by stochastic factors such as genetic drifts, bottlenecks, and founder effects but also by adaptation to regional difference in climate. In the Austronesian-speaking people who colonized Pacific islands within the last 6,000 years, the adaptation in energy expenditure to long voyage and life in isolated islands may influence their mtDNA variation. In this study, we examined association between mtDNA variation and physical indices such as BMI in Solomon Islanders. Sample collections and physical examinations were performed in three villages (Munda, Paradise, and Ravaki). The people living in the Ravaki village are immigrants from Kiribati in Micronesia. For typing of mtDNA, the D-loop region was sequenced and used as markers. We carried out stepwise multiple regression analyses considering sex, age, village, and mtDNA polymorphisms as variables. In the analyses, we found that sex, age, and village are significantly associated with BMI both in males and in females. In addition, an Austronesian-specific variant, 16247G, was significantly associated with lower male BMI ($P = 0.0282$). When only males under 40 years were considered, a stronger association was observed ($P = 0.0071$). Our results were unexpected since the 16247G is a major variant in Polynesian populations which have a high prevalence of obesity. Genetic adaptation of the Austronesians may be more complicated than we could expect.

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Mitochondrial and Y-chromosomal analysis of an indigenous Mexican population, the Filomeno Mata, to investigate admixture with the Spanish. *K.H. Ritchie[1], S. Harmon[1], R. Wiskirchen[1], J.E. Gomez[1], N.M. Myres[1], U.A. Perego[1], J.E. Ekins[1], L.A.D. Hutchison[1], L. Layton[1], M.L. Lunt[1], S.S. Masek[1], A.A. Nelson[1], M.E. Nelson[1], K.L. Pennington[1], J.L. Peterson[1], T.A. Tolley[1], S.R. Woodward[1,2].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; 2) Department of Microbiology, Brigham Young University, Provo, UT.

According to oral tradition, the Filomeno Mata population of eastern Mexico has remained in relative isolation since its initial encounters with Spanish conquers during the Conquistador era. Genetic analysis can support the legitimacy of such historical accounts as they are reflected in the extant genetic composition of a population. We report the estimated degree of European admixture present in the Filomeno Mata population based on concurrent analysis of Y-chromosome (Y-ch) and mitochondrial DNA (mtDNA). The differential histories of the maternal and paternal genetic records are also examined. Samples were collected from 141 females and 69 males from the Filomeno Mata village. All samples were typed for the characteristic Native American mtDNA haplogroups, A-D and X. Males were also tested using the Y-ch specific SNPs, DYS199, M242 and RPS4Y711. To further assess the degree of admixture, Y-ch haplogroups were then resolved into individual paternal lineages with 36 STR loci and compared with published Spanish and Portuguese datasets. The combination of mtDNA and Y-ch data demonstrate a complex genetic history of the Filomeno Mata.

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MOLECULAR ANALYSIS OF DUCHENE MUSCULAR DYSTROPHY AND SPINAL MUSCULAR ATROPHY GENES IN SAUDI PATIENTS. *M. Albalwi[1,3], M. Aljumah[2,3], I. Alabdulkareem[2,3].* 1) Division of Molecular Path & Genetics, Department of Pathology and Laboratory Medicine (1122); 2) Dept. of Medicine (#2216) Neurogenetics unit; 3) King Abdulaziz Medical City-Riyadh King Fahad National Guard Hospital P O Box 22490 Riyadh 11426 Saudi Arabia.

Molecular studies on neurological disorders among Arabs are scattered. In our laboratory, we have tested 483 Saudi patients referred for Duchene Muscular Dystrophy (DMD) or Spinal Muscular Atrophy (SMA). Our database of 124 patients referred because of suspected diagnosis DMD, gene deletion was detected in about 46 % (39/124). The DMD deletion was common in exons 4, 6, 12,13,17,19, 34, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52 and muscle promoter (Pm). No deletion was detected in exons 3, 32 and Brain promoter (Pb) as described in current protocols of human genetic (1994). However, 46.1% (18/39) had deletions in the know hot-spot region for both exons 51 and 52. Among 359 patients referred because of suspected diagnosis of SMA, homozygous deletion of exon 7 was detected in about 47% (115/244). On the other hand, our initial data showed that 3% of Saudis (3024 individuals were screened with an equal gender ratio) are carriers of SMA exon 7 gene deletion, a figure comparable to that of other international studies. However, the exact prevalence of SMA cases in Saudi Arabia is unknown but we expect it to be higher than international figures due to the high rate of consanguineous marriages.

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Accurate Polymorphism Detection in DNA Resequencing Data. *A. Quinlan, G. Marth.* Biology, Boston College, Boston, MA.

The discovery of sequence variants in individuals requires complete resequencing. This process typically uses PCR products from genomic DNA and results in diploid sequencing traces. Polymorphisms that appear as heterozygous bases must be detected within individual traces. Extant heterozygote detection tools such as POLYPHRED are useful, but have a high false positive rate, and do not provide reliable accuracy measures. As a result, laborious manual review is necessary.

Building upon our existing software POLYBAYES, we have devised a novel method of diploid polymorphism detection. Our design first detects heterozygous positions within each trace (diploid base calling), and then evaluates homologous base positions across every aligned trace. Diploid base calling is achieved with a support vector machine that allows us to classify unseen trace positions as either polymorphic or monomorphic. This determination and an associated probability of correctness are based on indicative features (e.g. peak height drop, secondary peak ratio) in appropriate training examples. We augment the usual features, which are provided by PHRED, with novel features of the peak shape from the binary trace files.

To determine whether a given position within the trace alignment represents a polymorphic site, we have modified the original Bayesian statistical scheme in POLYBAYES. This scheme takes into account the diploid base calls and corresponding probabilities calculated for each aligned trace, together with prior information such as the genomic or regional polymorphism rate, and the expected transversion/transition ratio to calculate the probability that the site in question is polymorphic.

Our initial results show that we are able to reduce both false positive and false negative rates significantly, as compared to existing software, and that the confidence scores for our calls are accurate. This is an important initial step in the redesign of POLYBAYES as a full-scale polymorphism discovery tool.

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Formalization of matching strategies for duplicated loci. *L.A.D. Hutchison[1], N.M. Myres[1], K.H. Ritchie[1], J.E. Ekins[1], J.B. Ekins[1], L. Layton[1], M.L. Lunt[1], S.S. Maesek[1], A.A. Nelson[1], M.E. Nelson[1], K.L. Pennington[1], U.A. Perego[1], J.L. Peterson[1], T. Tolley[1], S.R. Woodward[1,2].* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; 2) Dept. Micro and Molecular Biology, Brigham Young University, Provo, UT, USA.

Several commonly-used Y chromosome STR loci (including DYS385, DYS459 and YCAII) are referred to as duplicated, because PCR using the standard primers amplifies two different regions of the chromosome, potentially producing two alleles rather than one. As a result, it is not generally possible to unambiguously assign either allele to a locus copy, making it difficult to reliably compare the correct allele copies between two genotypes. Nevertheless, various strategies have been used in the literature to analyze data containing these these loci; for example, the total number of allele copies that match between two genotypes are sometimes counted as zero, one or two out of two matches. The various matching strategies in use do not technically capture the real properties of the data, and are therefore not completely accurate. Additionally, different matching strategies are not completely comparable, and error rates for the different strategies have not previously been determined. We present here a formalization of the matching of duplicated loci, and compare the theoretical error rates of different matching strategies from empirical data. We show that in certain situations, alleles can be compared unambiguously, and we derive a new matching strategy for duplicated loci from this result. We compare the accuracy of this new matching strategy to those used previously, and show we are able to significantly reduce error in matching duplicated loci. We discuss the impact of this improved matching strategy on the accuracy of calculations in forensics and population statistics. Extension of the method to matching of autosomal loci is discussed.

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Mutation spectrum of type I collagen genes in Korean OI patients. *K.S. Lee[1], T.M. Lee[1], H.S. Jin[1], H.Y. Park[1], S.C. Jung[2], S.K. Koo[1].* 1) Division of Genetic Disease, Department of Biomedical Sciences, National Institute of Health, Seoul, Korea; 2) Department of Biochemistry, College of Medicine, Ewha Womans University, Seoul, Korea.

Mutations in the type I collagen genes, *COL1A1* and *COL1A2*, are responsible for the dominantly inherited connective tissue disorder, osteogenesis imperfecta (OI). The severity of OI is diverse, ranging from perinatal lethality to a very mild phenotype with no deformity and normal stature. Although there are several studies of the mutational spectra of *COL1A1* and/or *COL1A2* in Western populations, only a few cases have been reported from Asia. In this study, we investigated 67 unrelated Korean probands with OI and used nucleotide sequence analysis to detect *COL1A1* and *COL1A2* mutations. Thirty-five different mutations in the two genes were identified, including 25 novel mutations. Among the 25 novel mutations, eight were glycine substitutions (G16R, G64D, G544S in COL1A1, and G235E, G268S, G586D, G730S, G766R in COL1A2), four were frameshift mutations in *COL1A1*, two were in-frame duplications in *COL1A2*, and eleven were splice-site mutations (six in *COL1A1*, five in *COL1A2*). The c.982 G to A mutation in *COL1A2* was found repeatedly, and was the causative mutation in 5 independent OI probands. The collagen gene mutation spectrum in Koreans may be representative of those in eastern Asian populations and its elucidation should broaden our understanding of the relationship between genotype and phenotype in OI patients.

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***CYP2D6* allele frequency in the Portuguese population.** *P. Santos[1], C. Correia[1], A.M. Coutinho[1], G. Oliveira[3], A.M. Vicente[1,2].* 1) Instituto Gulbenkian Ciência, Portugal; 2) Instituto Nacional Saude Dr Ricardo Jorge, Portugal; 3) Hospital Pediátrico Coimbra, Portugal.

Cytochrome P450 2D6 plays a critical role in the metabolism of about 20% of all prescribed drugs. The *CYP2D6* gene is highly polymorphic, with more than 80 allelic variants showing interpopulation differences. Alleles are classified, based on protein activity, into four different phenotypes: Poor (PM), Intermediate (IM), Extensive (EM) and Ultrarapid Metabolizers (UM). Determination of *CYP2D6* PM alleles *3, *4, *5, *6 and the UM gene duplication allows a correct prediction of enzyme activity, providing clinicians with a useful tool in drug prescription. The frequency of *CYP2D6* alleles *3, *4, *5, *6, and gene duplication was determined in 110 and 43 healthy Portuguese individuals, respectively. Allele frequencies were 0.014 (*3), 0.133 (*4), 0.028 (*5), 0.018 (*6) and 0.035 for the gene duplication, with five individuals classified as PM (4.9%) and three as UM (6.9%). Our results agree with previous studies in European populations: in Southern Europe frequencies for alleles *3, *4, *5, *6 and gene duplication vary between 0.007-0.03, 0.12-0.21, 0-0.034, 0-0.03 and 0.01-0.058, respectively, with 5-10% classified as PM and up to 7% as UM, while in Northern Europe the duplication is much rarer and the frequency of PM alleles is higher (0.23 for allele *4 and 0.04 for allele *5). The frequencies in Portugal reinforce the previously suggested north/south gradient of PM allele frequencies, which likely results from a North Africa anthropological influence in Southern Europe, especially in the Iberian Peninsula, as suggested by the low frequency of these alleles in African populations. Because the PM phenotype may result in toxic concentrations of CYP2D6 substrates with an increased risk of side effects, while the UM phenotype induces sub-therapeutic drug levels and thus lack of clinical effect, *CYP2D6* genotyping should be considered for drug prescription. Given the interpopulation *CYP2D6* heterogeneity, the determination of allele frequencies in specific populations is required for useful application of *CYP2D6* genetic information in clinical settings and in pharmacogenetic studies.

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The Construction of The Genetic Diversity Database of Chinese Populations. *J. Chu[1], T. Shi[2].* 1) Medical Genetics, Inst Med Biology, CAMS, Kunming, Yunnan, China; 2) Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031.

China is a country with a long history; it has over 13 billion people and 56 different ethnic groups. China offers abundant human genetic resources. To collect and reserve those resources for analyzing the genetic characteristics will push forward the study of disease genes and pharmacogenomics to a new level. From this point of view, China has the great unsubstituted advantages. Genetic diversity between different groups has long been an important part in Chinese human genome research. Under the support of the government and the efforts that the scientists have made, the genetic diversity research in China has achieved great success. A lot of information and data have been gathered. In order to collect these data and information regularly, and give a convenient way to future search and comparison and present information for related research fields, the Genetic Diversity Database has been constructed, which includes STR, mitochondrion DNA, HLA and SNP, as well as the information of the cell lines, the history of the populations and the information on each sample. At the same time, the database is linked to other related databases rich in related information, such as OMIM, dbSNP, HGMD, MITOMAP etc., and forms a knowledgeable database about the human genetic diversity. The users can retrieve a lot of information through the database. Beside that, the system has the interface for the data input and management. There are three different levels for the data to be shared in the database. According to the data owner's selection, the data can be shared in a lab, among the collaborating labs or released to the public. Each lab can set up the data sharing criterion by itself. The database can be reached through the web site: http://www.chgd.org.

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Using Single Nucleotide Polymorphisms (SNPs) to quantitate selection against nonsense, missense and silent mutations in humans. *I. Gorlov*[1], *M. Kimmel*[2], *C. Amos*[1]. 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Department of Statistics, Rice University, Houston, TX.

A large body of evidence suggests that point mutations in coding regions of the human genome are under purifying selection. If natural selection acts against point mutations in coding regions, then population frequencies of point mutations should be at equilibrium between a decrease in the frequency by purifying selection and an increase due to new mutations. According to an elegant population genetics formula, the equilibrium frequency of deleterious mutations depends on the selection coefficient and the mutation rate. Given reliable estimates of mutation rates we can estimate selection coefficients against different types of mutations if we know their equilibrium frequencies. To estimate equilibrium frequency of different types of point mutations in coding regions we have used NCBI dbSNP database on Single Nucleotide Polymorphisms (SNPs) in the human populations. We have adjusted proportions of SNPs for difference in codon usage and in mutation rates. We estimated selection coefficients against nonsense mutations, conservative missense, radical missesne, silent, and all missense mutations combined. We found that the average selection coefficient against missense mutations is ~12% of that against nonsense mutations. There are 3.5 fold differences in strength of selection against radical and conservative missense mutations. Surprisingly, we have found considerable selection against silent mutations - the selection coefficient is ~5% of that for nonsense mutations. Based on the estimated selection coefficients against nonsense, missense and silent mutations and mutation rate per nucleotide/per generation, we predict that average fitness drops ~5% each generation due to de novo point mutations in coding regions of human genome.

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Association of bone mineral density with a dinucleotide repeat polymorphism at the calcitonin (CT) locus in mexican population. *J. Magana Aguirre[1, 2], R. Gomez Ortega[2], L. Casas Avila[2], G. Suastegui[3], P. Diez Garcia[3], B. Cisneros Vega[1], M. Valdes Flores[2].* 1) Department of Genetics and Molecular Biology, CINVESTAV-IPN,07360 Mexico city, Mexico; 2) Department of Genetics, Centro Nacional de Rehabilitacion-SSA, 14389 Mexico city, Mexico; 3) Osteoporosis Clinic, Centro Nacional de Rehabilitacion-SSA, 14389 Mexico city, Mexico.

Osteoporosis is a multifactorial and poligenic disease, which is characterized by a generalized reduction in bone mineral density (BMD), and microarchitectural deterioration of bone tissue that results in an increased risk of bone fractures. Calcitonin (CT) may play a role in the pathogenesis of osteoporosis, genetics variations in or adjacent to the CT gene may be associated with variations in bone mineral density (BMD). The present study examined the correlation between a dinucleotide (cytosine-adenine) repeat polymorphism at the CT locus and BMD in 60 osteoporotic women, 60 non-osteoporotic women and 500 subjects of a Mexican general population. Nine alleles were present in the Mexican population. Alleles containing 10, 11, 12, 15, 16, 17, 18, 19 or 20 CA repeats. Thus, we designated the alleles as A, B, C, F, G, H, I, J and K respectively. We found that the A allele frequency was 63% in osteoporotic women, with a value significantly higher than that observed in non-osteoporotic women (35%) ($p<0.05$) and the AA genotype frequency was 45% in osteoporotic women, which was significantly higher than that found in non-osteoporotic women (18%) ($p<0.05$). For these reasons, the A allele and the AA genotype were related to the presence of osteoporosis (odds ratio 1.97 and 3.64 respectively). The loss of BMD and T scores (a parameter representing deviation from gender, race-adjusted average BMD) were compared between individuals that possessed one or two A alleles and those that did not posses the A allele. Subjects who possessed one or two A alleles had lower BMD and T scores than those who did not present any A allele (lumbar loss DMD: 26% vs 12% and T score: -2,3 vs -0.9). The significant association observed between BMD and genetic variation at CT locus implies that this polymorphism may be useful marker for the genetic study of osteoporosis.

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Comparison of two ancestral MHC DR3 haplotypes as a resource with which to examine common disease association. *A.N. Roberts[1], J.A. Traherne[2], R. Horton[3], M.M. Miretti[3], M.E. Hurles[3], J.L. Ashurst[3], P. Coggill[3], L.G. Wilming[3], J.A. Todd[1], J. Trowsdale[2], S. Beck[3].* 1) JDRF/WT DIL, CIMR, University of Cambridge, Cambridge, CB2 2XY, UK; 2) Dept Pathology, University of Cambridge, Cambridge, CB2 1QP, UK; 3) WT Sanger Institute, Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

The major histocompatibility complex (MHC) is a highly duplicated and polymorphic region of the human genome on chromosome 6p21.31 and its core spans ~4 Mb including > 150 expressed genes. The aim of this project is to sequence and analyze the total DNA content and polymorphisms of the MHC region from eight consanguineous HLA-homozygous cell lines (http://www.sanger.ac.uk/HGP/Chr6/MHC/). Here, we present 4.25 Mb of the third haplotype, QBL (HLA-A26, -B18, -Cw5, -DR3, -DQ2) and compare the findings with that from the first two haplotypes, PGF (HLA-DR2(15)) and COX (HLA-A1, -B8, -Cw7, -DR3, -DQ2), (http://vega.sanger.ac.uk). Both QBL and COX share the classical serological determinant, DR3, and, therefore, some commonality in their origin. We have defined this shared segment of DNA identical-by-descent to only a 158 kb-region between *HLA-DRB3* and *HLA-DQB3* which contains the DR3 serological determinants *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1*. The time to the most recent common ancestor (MRCA) is estimated to be less than four thousand years. Outside this segment, the divergence between QBL and COX is as extensive as we found previously between PGF and COX: we identified ~15,000 SNPs, of which 6,159 were novel to this QBL project and ~2,000 DIPs. Most of the SNP variation was concentrated in peaks overlying the classical class I and class II loci. The heterozygosity per nucleotide site () between any two MHC haplotypes in this study falls in the range of 2.7 - 3.6 x 10^{-3}, which is 4-7 fold higher than typical genomic wide diversity. Delineation of the boundaries of the hypervariable *HLA-DRB* region is defined using genomic repeat elements as handles. The sequences of the remaining haplotypes will not only reveal further polymorphisms for genetic dissection of the MHC in disease but also define ancestral relationships between haplotypes.

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An unusual haplotype structure at inversion polymorphic region on human chromosome 8p23. *L. Deng[1], T. Liu[2], H. Zhao[1], J. Kang[3], H. Zhao[1], T. Niu[4], H. Yang[1], C. Zeng[1], BGI HapMap Group.* 1) Beijing Genomics Institute, Chinese Academy of Sciences, Beijing, China; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; 3) Department of Mathematics, Beijing Normal University, China; 4) Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Using population genotype data of 1072 single nucleotide polymorphisms (SNPs) generated by the HapMap project, we delineated genomic structure of a previously reported 3.8-Mb inversion polymorphism region at chromosome 8p23 (Giglio et al. 2001). Analysis of molecular variance and pairwise FST comparison of individual SNPs revealed great genetic differentiation among populations. Divergent population subgroups were resolved in CEU samples under the ¡admixture model¡ of the structure. Considering this inversion segment as a specific polymorphism frame, three distinct haplotype clades, named as Clade_1, Clade_2, and Clade_3, were detected based on the phylogenetic clustering analysis. The distribution of these clades among geographic populations was considerably different. Clade_2 was mainly detected in CEU samples and Clade_3 was only seen in YRI samples. Clade_3 was identified as the ancestral type evident by its co-clustering with chimpanzee haplotype in phylogenetic analysis. For future larger-scale studies of genetic association and human evolution in this region, tag SNPs were also selected based on principal component analysis and greedy-discard method. Further analysis regarding the large dissimilarity of genetic background among geographic populations resulted from different inversion frequency, as well as cytogenetic localization of this inversion polymorphic region by fluorescent in situ hybridization, would also be presented. Taken together, our results provide a panoramic view on structural and population features of the inversion polymorphism on 8p23, as well as a primary tool to detect and deduce the orientation of chromosomal arrangement.

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Linkage Disequilibrium and haplotype structure in the *DC-SIGN* gene in Caucasians and Afro-Caribbeans. *J. Martinson[1,2], Y. Li[2], R. Bosko[1], R. Ferrell[2].* 1) Inf Diseases & Microbiology, Univ Pittsburgh, GSPH, Pittsburgh, PA; 2) Human Genetics, Univ Pittsburgh, GSPH, Pittsburgh, PA.

The Dendritic Cell protein DC-SIGN plays a pivotal role in the early stages of the immune response, as it is involved in both pathogen peptide processing and Dendritic Cell motility. Many pathogens exploit DC-SIGN, either as an adhesion receptor in the infection of Dendritic Cells, or as a means of escaping the host immune response. The *DC-SIGN* gene is a member of the *CD209* gene family: genes in this family are heavily alternately spliced, generating variant forms of their protein products that differ in cell tropism and cellular localization. Genetic variation in *DC-SIGN* may therefore be of importance in disease resistance and immune function.

In an earlier study, we reported initial data on nucleotide variation in *DC-SIGN* in Caucasians, and here present results of a more extensive survey of SNPs and haplotypes in this gene in Caucasians and in sub-Saharan African-descended inhabitants of the Caribbean island of Tobago. We used a combination of DHPLC and resequencing to obtain SNP data on coding, non-coding and regulatory sequences averaging 6.0kb per person for 120 Caucasians and 96 Tobagans.

We observed a high degree of LD in Caucasian samples. Although we identified 48 SNPs in Caucasians, 34 of these were rare (minor allele frequency <10%). Determination of the likely haplotypes present, using the PHASE software package, showed a total of 74 haplotypes based on 48 SNPs, but only six of these haplotypes accounted for 50% of the samples. Analysis of LD parameters using the ldmax and GOLD packages showed high LD extending across the whole of the gene. In contrast, LD in Tobagans was very patchy, with small blocks of LD in the promoter and downstream regions, but no LD between these blocks. Tobagans were also characterised by a higher level of haplotype diversity than Caucasians, including haplotypes based on SNPs not observed in Caucasians. The differing demographic histories of these populations has had a profound effect on LD and haplotype structure at the *DC-SIGN* gene.

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Evidence for Constraint in Telomere Stability Genes. *S.A. Savage[1], M. Yeager[2,3], J. Liao[1], S.J. Chanock[1].* 1) Pediatric Oncology Branch, CCR, NCI, NIH, Bethesda, MD; 2) SAIC-Frederick, Bethesda, MD; 3) OD, DCEG, NCI, NIH, Bethesda, MD.

Telomeres, nucleotide repeats at the ends of chromosomes, are essential for chromosomal integrity. An ordered protein complex at the telomere protects chromosomes from end to end fusion. Previously, we reported that the pattern of genetic variation in 7 key telomere genes, *POT1, TERT, TERF1, TERF2, TINF2, TERF2IP* and *TNKS*, showed limited nucleotide diversity, a paucity of nonsynonymous single nucleotide polymorphisms (SNPs) and variability in linkage disequilibrium (LD) in 4 ethnic groups (n=118 individuals divided between AA-African/African-American, CA-Caucasian, HI-Hispanic, PR-Pacific Rim). In this study, the analysis was extended to include SNPs in *POT1, TERT, TERF1*, and *TINF2* in an additional set of 280 individuals from the CEPH Human Diversity Panel (76 AA, 66 CA, 49 HI and 89 PR), which is large enough to effectively estimate haplotype diversity. Overall, SNP frequencies were comparable and limited haplotype diversity was again observed. LD in *TERT* is particularly limited whereas there is strong LD across *POT1*. Population specific SNPs were observed in *TINF2*. Bi-directional sequence analysis was performed in two additional genes, *PTOP* and *PINX1*, in 24 individuals from 3 ethnic groups to identify common variants. Sequence analysis included all exons, 5 and 3 UTRs as well as regions of homology between human and mouse sequences. Haplotypes were inferred and LD was estimated. This analysis suggests that common variants are rare in *PTOP* and *PINX1*. Haplotype structure was restricted and slightly more complex in AA than CA, HI and PR and LD was variable. Studies are underway to characterize the extent of variation and LD in *PTOP* and *PINX1* in larger sets of individuals. In summary, telomere stability genes appear to be constrained; most likely due to the role they play in chromosomal stability. Thus, genetic variation in telomere stability genes suggests that they could be suitable candidate genes for study in autoimmune, cancer and hematologic disorders.

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Gene Copy Number Quantitation in Drug Metabolism Genes by Real-Time Quantitative PCR. *S. Liew, K. Lazaruk, L. Wong, J. Stevens, K. Livak.* Applied Biosystems, Foster City, CA.

Polymorphisms in drug metabolism genes have been classically identified as SNPs, indels and STRs. Gene deletion and gene duplication are genomic variations that can also affect enzyme function or phenotype. Deletions and duplications in drug metabolism genes have been associated with phenotypic variation, for example, they can characterize a phenotype as poor (PM), intermediary (IM), extensive (EM), and ultrarapid metabolizer (UM). Gene copy number, or gene dosage, can be accurately determined using real-time quantitative PCR. The method involves relative quantification of the gene of interest versus a reference gene known to be single copy. Relative gene copy number is determined by the Ct method, a well-established relative quantitation method used in mRNA quantitation studies. We have developed assays to measure gene dosage in a variety of genes, including drug metabolism genes such as CYP2D6 and GSTM1.

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Y-CHROMOSOM POLYMORPHICS MARKERS IN INDIGENOUS GROUPS Of THE ZULIA STATE. *T.C. Pardo[1], L. Borjas[2], W. Zabala[2], G. Portillo[2], J. Marquez[2], A. Aranguren[3].* 1) Laboratorio de Genética Molecular. Unidad de Genética Médica. university of zulia, Maracaibo, Venezuela; 2) Instituto de Investigaciones Biológicas, university of zulia, Maracaibo, Venezuela; 3) Cátedra de Genética. Facultad de Ciencias Veterinarias. Universidad del Zulia, Maracaibo, Venezuela.

The first natives that populated Zulia state, derived from three main linguistic groups: Arawacos (Wayúu), Caribbean (Yukpa) and Chibchas (Barí). The Wayúu were the first ones accepting the European colonization, assimilating some of their customs and having marriages among them, contrary to the observed in Barí and Yukpa, culturally closed groups, geographically isolated, where the endogamy prevailed. To characterize the genetic structure of three indigenous groups through the analysis of DYS385a/b, DYS389I, DYS389II, DYS287 and DYS199 in human Y chromosome. Four loci Y-STRs, the DYS199 and the insert Yap Alu were analyzed by PCR in a sample of 71 individuals belonging to the 3 mentioned groups. Thirty two (32) different haplotype was observed; twenty-seven (27) in Wayúu and five (5) in Barí, the Yukpa presented an only haplotype that repeated in the Wayúu. For each locus, the levels of genetic diversity varied between 0 and 0.72 and those of haplotipc diversity between 0 and 0.96. the AMOVA test showed a bigger intra-population variation (66.95%) that inter-populations (33%). DYS389II presented high frequencies for different groups, showing that there is a genetic difference among these groups. As for the assessment of mixture degree, Barí and Yukpa showed an Amerindian mono-parental contribution while in the Wayúu the Amerindian component was followed by the European one. The Y-STRs haplotypes constitute a very useful tool for the molecular anthropology studies, since it allows to study the genetic relationships and composition of different populations.

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Application of a DNA microarray based comparative genomic hybridization (Array-CGH) method to the study on the genetic effects of atomic bomb radiation. Results from a pilot study. *N. Takahashi[1,4], K. Sasaki[1], M. Kodaira[1], Y. Satoh[1], Y. Kodama[1], K. Sugita[2], H. Katayama[2], N. Tsuyama[3].* 1) Dept Genet, Radiation Effects Res Fdn, Hiroshima, Japan; 2) Dept Inf Tech, Radiation Effects Res Fdn, Hiroshima, Japan; 3) Dept Bio-Signal Anal, Grad Sch Med Yamaguchi Univ, Ube, Japan; 4) CREST, Kawaguchi, Japan.

[Purpose] We have studied the effects of A-bomb radiation on human germ cells, that is, whether the mutation rate has increased significantly in the offspring of A-bomb survivors compared with controls at the genomic DNA level. To enable genome-wide detection of these mutants, we have been introducing a microarray based comparative genomic hybridization (array CGH) method. Before starting a large-scale study, we conducted a pilot study to examine the feasibility of this method. [Experiments] We constructed an array where about 2400 Bac-clones were printed onto three different slide glasses as one set. These clones are distributed about every 1.2 Mb across human autosomes. After preliminary studies for establishing good experimental conditions, we did a population study on 40 offspring of A-bomb survivors and 40 members of a control group. [Results and discussion] Fourteen variants were observed once in this population, termed rare variants, and the results from family studies revealed that these variants were inherited from at least one parent. Thus, until now, no de novo mutant was identified. We characterized these rare variants by Southern blot analysis using pulse field gel electrophoreses, quantitative PCR, FISH method, and sequencing. These results demonstrated that our array CGH system can reliably detect the variants larger than 50 kb. Moreover, 18 kinds of variants were recurrently identified in this population, and they were termed polymorphic variants. Southern analyses conducted for these polymorphic variants demonstrated that many showed various complicated patterns. From our data, we consider that array CGH is one of the most useful techniques for our research purposes. Now, we are planning a larger population study.

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Sequence variation at the GABA transporter-1 gene (*SLC6A1*) in five populations. *R. Hirunsatit[1,2], R.L. Malison[1], P. Räsänen[4], A. Sughondhabirom[2], N. Thavichachart[2], S. Tangwonchai[2], A. Mutirangura[2], H. Kranzler[5], J. Gelernter[1,3], J. Lappalainen[1,3].* 1) Psychiatry, Yale University, New Haven, CT; 2) Chulalongkorn University, Bangkok, Thailand; 3) VA Connecticut Healthcare System, West Haven, CT, USA; 4) University of Oulu, Department of Psychiatry, Finland; 5) University of Connecticut, School of Medicine, Department of Psychiatry.

The *SLC6A1* gene encodes the -aminobutyric acid (GABA) transporter-1, which participates in the regulation of synaptic GABA levels via re-uptake of GABA. GABA transporter-1 is the selective therapeutic target of tiagabine, a novel anticonvulsant medication. The goal of this study was to identify sequence variation and the haplotype structure of *SLC6A1* in different populations, representing three of the major continental groups, in anticipation of pharmacogenetic studies of tiagabines therapeutic and adverse effects. Approximately 11.6 kb of the *SLC6A1* locus were sequenced, including the 5promoter region, all 16 exons, and large segments of the flanking intronic regions, in eight samples from Finnish, Thai, Hmong, European-American (EA), and African-American (AA) subjects (n=40). A total of 55 SNPs were found in these samples; 20 were present in more than one population studied. Population-specific SNPs were rare in the Finnish, Thai, and EA samples. Common population specific SNPs were discovered only in the AA sample (p>0.25). Twenty seven SNPs were novel (not reported in the NCBI SNP database). No non-synonymous SNPs were identified. Nucleotide diversity () in *SLC6A1* was highest in the AA population. There were no marked differences in between the EA, Finnish, Thai and Hmong populations. To evaluate the linkage disequilibrium (LD) structure of *SLC6A1*, 11 SNPs were genotyped in 47 Finnish, 41 Hmong, 46 EA, 46 AA, and 46 Thai subjects using the 5 nuclease (Taqman) method. Preliminary analysis indicated that the LD structure of *SLC6A1* is similar in the five populations we studied. These findings from extensive resequencing and haplotype analysis of the *SLC6A1* gene will be useful for pharmacogenetic studies focusing on the therapeutic and adverse effects of tiagabine and other medications that influence GABA transporter-1 function.

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Association of TNF alpha gene -308 GG genotype with post operative infections in esophageal cancer patients. *K. Azim, K. Brophy, R. McManus, D. Kelleher, J.V. Reynolds.* Inst Molecular Medicine, Trinity Col Dublin and Dublin Molecular Medicine Centre, Dublin, Ireland.

Purpose: To find the effect of TNF alpha gene biallelic single nucleotide polymorphism (SNP) at -308 position on post operative infections in Esophageal Cancer patients. Methods: In a retrospective case-control study design, we selected 197 Esophageal Cancer patients who underwent surgery in St. Jamess hospital, Dublin Ireland. The patients were divided in three groups; 1) Post Operative (PO) Infections (55); 2) No PO complication (114); and 3) Other PO complications (28). DNA was extracted and genotyping was done using Taqman chemistry and PCR/RFLP. Relevant clinical data was obtained for all study subjects and possible confounding patient characteristics were taken into account during statistical analysis. The study had local Ethical Committee approval and informed consent was obtained from all study subjects. Results: The distribution of GG homozygotes was significantly higher in the Post operative infection group ($P = 0.021$), and G allele was significantly higher in post operative infection group as compared with no complication group ($p = 0.017$) and other complication group ($p = 0.013$). No significant difference was observed in the three groups as regards preoperative chemotherapy, tumor stage, differentiation, location and histology, though there was a significant difference in the age of the patients, the post operative infection group being older. Conclusion: TNF alpha gene -308A allele has been shown to be associated with higher circulating levels of TNF alpha and the -308 G allele is a comparative low secretor allele. Many recent studies have reported a higher incidence of infections in patients under anti TNF alpha therapy (infliximab or etanercept). We propose that the polymorphism in the promotor region of TNF alpha gene may lead to altered expression and a possible suboptimal activity of TNF alpha in GG genotypes, predisposing them to a higher risk of developing infections.

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P-gene variability in North Eurasian human populations. *M.A. Perkova, B.A. Malyarchuk, M.V. Derenko.* Genetics Laboratory, Institute of Biological Problems of the North, Magadan, Russian Federation.

To estimate the level of variation of the P-gene, the human homologue to the mouse pink-eye dilution locus, polymorphism at three loci (R305, A355, and R419) was studied in 350 normally pigmented individuals representing different populations of North Eurasia. These samples were grouped into four ethnogeographic areas: Europe (the Russians), Southwest Asia (the Iranians), South Siberia (the Buryats), and Northeast Siberia (the Chukchi, Koryaks and Evens). We found that the heterozygosity level ranges from 54 per cent in Europe and 53 per cent in Southwest Asia to the lower values of 49 and 30 per cent in South Siberia and Northeast Siberia, respectively. Differences among regional groups account for 1.7 per cent of the total variation ($p = 0.003$); however, in pairwise comparisons significant Fst-differences were revealed only between Northeast Siberians and other regional groups studied. In addition, the results of the present study show that there are significant differences between distribution of R419G variant in Russian populations ? the 419G allele frequency is much higher in the northwestern Russians (6.2 per cent in Velikij Novgorod sample) than in southwestern ones (3.6 per cent in Belgorod sample). Interestingly, previous studies have indicated that increased proportion of 419G alleles appears to be characteristic for individuals with green/hazel eyes compared with others (Rebbeck et al. 2002). Thus, the results of our study suggest that allelic variation in the P-gene may be associated with normal variability in pigmentation of the eyes, hair and skin at the population level. This work was supported by the grant from the Far-East Branch of the Russian Academy of Sciences (05-3--06-096).

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Association of Uteroglobin related protein (UGRP)1 gene polymorphism with Asthma in African Americans and Caucasians. *K. Yanamandra, E. Chen, D. Napper, P.B. Boggs, S.A. Ursin, H. Chen, J.A. Bocchini Jr., R. Dhanireddy.* Dept Pediatrics, LSU Medical Ctr, Shreveport, LA.

Asthma is a multifactorial, polygenic disorder involving the contribution of many genes and the environment. Because of its polygenic inheritance, many investigators studied the role of several genes including those of interleukins and non-interleukins. One of the genes studied in the Japanese population was Uteroglobin related protein (UGRP)1 gene, encoding the UGRP1 protein which was thought to be an anti-inflammatory agent. A polymorphism in the promoter region of the gene, carries a SNP transition from G to A at position 112 which results in 24% reduction in the promoter activity compared to the wild type. UGRP1 is a secretory protein mainly expressed in the lung and trachea and has recently been implicated in asthma. Although Niimi T, et al. in 2002 have shown an association of -112A allele with asthma in Japanese population, Jian Z, et al. in the following year in 2003, contradicted the association, in another Japanese population with childhood atopic asthma. And, the findings of Jian Z, et al. were confirmed by Batra J, et al. in 2005, in the Indian asthmatic population. We, at the Molecular Genetics Laboratory at LSU Health Sciences Center in Shreveport, Louisiana, have been studying the role of several genes in asthma for the past several years. In the present investigation, we began to study the role of UGRP1 gene in the etiology of asthma by genotyping 120 asthmatic individuals and 250 control individuals, from Northwest Louisiana population. The -112A allele frequency was same in both patients and controls at 0.16, among the Caucasians. Similarly, the -112A allele frequency was 0.35 in patients and 0.30 in controls among the African Americans. Our data does not indicate an association of UGRP1 polymorphism with asthma in either African Americans or in Caucasians. This is the first report in the literature to show the role of this polymorphism in asthmatic patients in the African American community. Data, statistics, and distribution of genotypes with ethnic break down of patients and controls will be presented.

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The -globin recombinational hotspot reduces the effects of strong selection around HbC, a recently arisen mutation providing resistance to malaria. *E.T. Wood[1,2], D.A. Stover[2], M. Slatkin[3], M.W. Nachman[1], M.F. Hammer[1,2].* 1) Department of Ecology & Evol Biology; 2) Division of Biotechnology, University of Arizona, Tucson AZ; 3) Department of Integrative Biology, University of California, Berkeley, CA.

Recombination is expected to reduce the effect of selection on the extent of linkage disequilibrium (LD), but the impact that recombinational hotspots have on sites linked to selected mutations has not been investigated. We empirically determine chromosomal linkage phase for 5.2-kb spanning the -globin gene and hotspot. We estimate the HbC mutation, which is positively selected due to malaria, originated less than 3,000 years ago and that selection coefficients are 0.04-0.09. Despite strong selection and the recent origin of the HbC allele, recombination (crossing-over or gene conversion) is observed within 1-kb 5 from the selected site on more than one third of the HbC chromosomes sampled. The rapid decay in LD upstream of the HbC allele demonstrates the large effect the -globin hotspot has in mitigating the effects of positive selection on linked variation.

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An Updated World-Wide Characterization of the Cohen Modal Haplotype. *J.E. Ekins[1], E.N. Tinah[2], N.M. Myres[1], K.H. Ritchie[1], U.A. Perego[1], J.B. Ekins[1], L.A.D. Hutchison[1], L. Layton[1], M.L. Lunt[1], S.S. Masek[1], A.A. Nelson[1], M.E. Nelson[1], K.L. Pennington[1], J.L. Peterson[1], T. Tolley[1], S.R. Woodward[1,2].* 1) Sorenson Molec Genealogy Fndn, Salt Lake City, UT; 2) Department of Micro and Molecular Biology, Brigham Young University, Provo, UT.

Since the definition of the Cohen Modal Haplotype (CMH) in 1998, the 6 SNP-6 STR genetic motif has been utilized to infer connections of contemporary individuals and communities to the ancient Hebrew population. The elucidation of the YCC SNP Phylogeny has allowed cataloguing of chromosomes compatible with the original CMH definition into several different Y-SNP subclades. Haplogroup membership was determined for 266 samples matching at 5 of the CMH STR alleles, defined as the Cohen Modal Haplogroup (CMHg). The bulk of the CMHg chromosomes were observed in J1 (53.0%) and J2 (43.2%), with a small portion falling outside of haplogroup J (3.8%). Members of the CMHg were observed throughout the world, with significant frequencies in various Arab populations: Oman (20.1%), Iraq (15.2%), Palestine (9.5%). Coalescent simulations were performed for CMH chromosomes within each SNP haplogroup using 24 STR loci. Estimates within J1 [6.5kybp(4K-12K)] and J2 [13kybp(7K-27K)] were substantially deeper than previous figures obtained from a heavily weighted Jewish sampling, indicating a likely origin of the compound haplotype prior to the establishment of the Hebrew population. The significant presence of CMH chromosomes in deeply divergent clades J1 and J2 (>20kybp), indicates the present CMH definition is not sufficient to distinguish lineages that likely arose by parallel IBS mutations. An expanded STR definition is proposed which allows differentiation between CMH-compatible chromosomes in J1 and J2. The inference of Jewish ancestry based on the original CMH definition should be performed with caution as subjects may be falsely categorized into the eponymous CMH lineage when the true origin is in the deeply divergent IBS branch. These observations underscore the importance of using updated SNP classifications when utilizing the CMH to infer ancestry in Jewish populations, or the use of the expanded STR definition.

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Genomic regions exhibiting positive selection identified from dense genotype data. *C. Carlson[1], D. Thomas[2], M. Eberle[1], R. Livingston[1], M. Rieder[1], D. Nickerson[1].* 1) Dept Genome Sci, Univ Washington, Seattle, WA; 2) University of California, Santa Cruz, CA.

Patterns of nucleotide diversity can be used to test for signatures of natural selection in sequences that depart from the expected distribution of nucleotide diversity under the neutral theory. We observed a significant ($p<0.001$) correlation between the Tajima's D test statistic in full resequencing data and Tajima's D in a dense, genome wide data set of genotyped polymorphisms for a set of 178 genes. Based on this, we used a sliding window analysis of Tajima's D across the human genome to identify regions putatively subject to strong, recent selective sweeps. This survey identified seven Contiguous Regions of Tajima's D Reduction (CRTRs) in African Americans (AD), 23 in European Americans (ED), and 29 in Chinese Americans (XD). Only four CRTRs overlapped between populations, three between ED and XD, and one between AD and ED. Full resequencing of six genes within four CRTRs demonstrated patterns of nucleotide diversity inconsistent with neutral expectations for at least one gene within each CRTR. Identification of the functional polymorphism (and/or haplotype) responsible for the selective sweeps within each CRTR may provide interesting insights into the strongest selective pressures experienced by the genome over recent evolutionary history.

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How frequently is common variation shared among US populations? An unbiased assessment based on 3,873 resequenced genes. *S. Guthery[1], B. Salisbury[2], M. Pungliya[2], C. Stephens[3], M. Bamshad[1,4].* 1) Dept of Pediatrics, Univ of UT, SLC, UT; 2) Genaissance Pharmaceuticals, New Haven, CT; 3) Motif BioSciences, NY, NY; 4) Dept of Human Genetics, Univ of UT, SLC, UT.

The common variant/common disease hypothesis posits that genetic susceptibility to complex diseases is influenced mainly by variants that are shared among populations. Analyses of allele sharing have shown that most common (ie, 10% in a given group) variants are shared among African-, Asian-, and European Americans. In most of these studies, alleles were measured by genotyping variants ascertained as common in one or a few populations, which can upwardly bias estimates of allele sharing among groups. To obtain an unbiased estimate of allele sharing, we examined resequencing data from the regulatory and coding regions of 3,873 genes in individuals self-identified as Hispanic (n=17), African- (20), Asian- (19), and European Americans (20). A total of 63,012 variants were found. Six pairwise population comparisons of variants with frequencies 10% showed that 71-96% of variants common in one population were present in both populations, though only 44-72% of such variants were common in both populations. Correlation of the frequency of common variants between populations ranged from 0.23 in African Americans vs. Hispanics to 0.83 in European Americans vs. Hispanics. Correlations were consistently lower in comparisons with African-Americans. Approximately 60% of variants common in at least one population were present in all populations, but only 32% of them were common in all populations. These results indicate that a variant common in one population is, in general, present in other populations but not necessarily common in other populations. Moreover, common variants are frequently not shared between African Americans and non-African populations. These findings suggest that a sizable fraction of genetic factors that influence common health-related traits might vary substantially between populations, particularly Africans vs non-Africans. It would therefore be prudent to develop more initiatives to identify common variation and risk variants in non-European populations, particularly African Americans.

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Ethnic admixture in Maracaibo, Venezuela: Analysis based on biparental and uniparental DNA markers. *L.M. Pineda Bernal, L. Borjas, W. Zabala, C. Castillo, M.G. Portillo, M. Mendt, E. Fernández, M.A. Sánchez.* Laboratorio de Genética Molecular.Unidad de Genética Médica. Facultad de Medicina. Universidad del Zulia, Maracaibo, Zulia, Venezuela. Apartado Postal 4002.

According with historical records the population of Maracaibo, at the northwest of Venezuela, is considered to be a result of the mating of European and African derived people with the local Amerindians, but the precisely contribution of each one is needed to be established with a several DNA markers. Objective: We analyzed in this study several DNA polymorphisms to clarify about the genetic structure and ethnic admixture of the contemporary Maracaibo population. Material and methods: DNA was extracted from blood samples of individuals born and living in Maracaibo after signing an informed consent. The blood samples were analyzed by PCR, capillary electrophoresis and/or PAGE for the following genetic markers: VNTR D1S80 and fifteen autosomal STRs, two biallelic polymorphisms of Y chromosome, twelve Y-STRs and the Amerindian mt-DNA haplogroups. Intra and interpopulations parameters were obtained with ARLEQUIN, DISPAN, and ADMIX3 softwares. We used as parental groups the published data from European, African and Amerindian populations. Results: The results with biparental polymorphism and Y-markers reveal that Maracaibo sample has a main European genetic contribution (more than 80%), followed by Amerindian and African contribution. However, in data derived from mt-DNA haplogroups showed an important Amerindian component in the sample (50%). These results suggest the presence of an asymmetric patterns of mating with a predominant contribution of European males with Amerindian females. These results are in accordance with historical records about the predominance of colonizer males, the almost extermination of the males natives and limited entrance of African people into Maracaibo population.

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Investigation of YSTR haplotype clustering among Caucasians in the US. *D. Garvey[1], P. Berdos[2], L. Sims[2], J. Ballantyne[2].* 1) Department of Physics, Gonzaga University, Spokane, WA; 2) Center for Forensic Science, University of Central Florida, Post Office Box, 162367, Orlando, FL 32816-2367.

An analysis was conducted of 17 YSTR haplotypes for 298 randomly sampled Caucasian men in the US. The approach used was to treat each of the 17 alleles as a coordinate in a 17 dimensional space, so that each haplotype was represented as a lattice point in that 17 dimensional space. This analysis found that 85% of the haplotypes were grouped into one of three isolated clusters, with the population density at lattice points outside those clusters being nearly zero. SNP genotyping identified these three clusters as being separately composed of men belonging to haplogroups R-M269, I-M253, and R-M17 (in order of decreasing cluster membership). The highest population density of each haplogroup cluster occurred at the lattice point at the center of the cluster, and all three clusters were seen to have more than 50% of the cluster member haplotypes differing from the central lattice point by three or fewer repeat unit steps. This clustering of haplotypes means that the forensic usefulness of YSTRs must be evaluated based on intra-cluster variation, as opposed to evaluating the diversity seen in the whole population. The most extreme example of this was DYS438. DYS438 showed a whole population variance of 0.90, but was seen to be nearly monomorphic inside each cluster.

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mtDNA diversity and the settlement of the Peruvian Andean Altiplano. *N.M. Myres[1], J.E. Myres[1,2], M. Malan[2], J. Fuller[2], M. McNairy[2], M.J. Rowe[2], R.T. Matheny[2], H.A. McCammon[1], S.R. Woodward[1,2].* 1) Sorenson Molec Genealogy Fndn, Salt Lake City, UT; 2) Department of Microbiology, Brigham Young University, Provo, UT.

A more adequate understanding of New World settlements and biologic history is accessible within the context of selected population studies. Examination of regional populations will more completely address the nature of New World settlement within the framework provided by broader studies. Selected regional studies can yield a more refined assessment of settlement scenarios that include intra-continental migration because of the different developmental history of each group. The current study examines the population structure of Andean Altiplano populations from the Lake Titicaca Basin within the context of South American populations. We report the mtDNA haplogroup distribution of 454 samples from island and mainland populations of the Lake Titicaca region and identify control region sequence polymorphisms of 58 individuals. A selectively dispersed mtDNA control region motif is also presented as a diagnostic marker for tracing gene flow and founding events within the Lake Titicaca region and surrounding areas.

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Genetic admixture, phenotype, and perceived admixture in Hispanics and Native Americans in New Mexico. *Y. Klimentidis.* Anthropology, University of New Mexico, Albuquerque, NM.

Examining admixed populations is useful for disease mapping, characterizing inter-population differences, and for exploring interactions between genetic admixture and various personal, social, and phenotypic outcomes such as BMI, % body fat, and skin pigmentation. This study considers a population of Hispanics and Native Americans from the state of New Mexico. A panel of AIMs (Ancestry Informative Markers) is used to classify individuals along the European-Native American ancestry spectrum. Measurements of skin pigmentation, height, weight, % body fat as well as facial photographs are obtained from each individual. In addition, various data on self-identified ethnicity, self-perceived admixture proportions, social environment, and familial history is obtained. Relationships between genetic ancestry and self-perceived ancestry/ethnicity are examined as well as the perception of others degree of shared ancestry as judged from facial photographs where both respondent and photographed individual have known degrees of genetic admixtures. Results generally show that genetic admixture is a moderately good predictor of a variety of traits, and that these are influenced by social and cultural factors. Implications concerning human evolutionary ecology and human diversity are discussed.

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Evidence of Overdominant Selection for IL-10 Receptor (IL10RB) and IFN- Receptor 2 (IFNGR2) in European Americans but not in African-Americans. *N. Wang[1], X. Sheng[2], R. Chakraborty[2].* 1) Divs. of Allergy/Immunology and Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Recent studies have indicated that human disorders mediated by T-helper 1 (TH1) and T-helper 2 (TH2) pathways, such as type-1 diabetes and asthma, could down-regulate each other. IL-10 receptor (IL10RB) and IFN- Receptor 2 (IFNGR2) are required for IL-10 and IFN- to active the receptor complex, respectively. IFN- is mainly responsible for the TH1 response; IL-10 plays an important role by suppressing TH1-dependent but supporting TH2-dependent immune response. Overdominant selection (heterozygote advantage) suggests that heterozygous individuals at particular loci have a higher fitness than homozygotes. Our analyses of the resequence of IL10RB and IFNGR2 genes in 24 European Americans (EA) and 23 African-Americans (AA) show that the allele frequency and heterozygosity distributions of polymorphisms in these two genes are considerably different from those of selectively neutral genes in EA. A large proportion of the single nucleotide polymorphisms (SNPs) in IL10RB and IFNGR2 have intermediate frequencies; approximately 65% and 52% of the heterozygosity of IL10RB and IFNGR2, respectively, are contributed by such intermediate SNPs. These results suggest that IL10RB and IFNGR2 genes might be influenced by overdominant selection in EA. Since the evidence for overdominant selection could be confounded by population demographic history, such as population bottleneck, we conduct coalescence simulation for these two genes. Our simulation results rule out population bottleneck as the explanation, and support that overdominant selection is the primary cause. Interestingly, the evidence for overdominant selection of these two receptors is not observed in AA, suggesting that those SNPs with intermediate frequencies in IL10RB and IFNGR2 might be maintained after human moved out of Africa as an adaptation to local surroundings. The results obtained from our study provide new clues and emphasize the importance of these two gene receptors in diseases related to TH1 and TH2 pathways.

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The efficiency and transferability of tagSNPs in six populations from three tagSNP selection algorithms. *S. Gu*[1], *N. Liu*[2], *H. Zhao*[1,2], *K.K. Kidd*[1]. 1) Department of Genetics; 2) Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

The HapMap project is focused on three continental groups, Yoruba from Africa, Mixed Europeans from Utah, and two Asian populations. However, there are few data that address how useful tagging SNPs selected on one population will be in another population from the same continent. Our analyses to test the generalizability of tagging SNPs across populations have used samples of two African populations (Yoruba and Hausa), samples of two European populations (Irish and Danes), and samples of European Americans and African Americans. The data consisted of 247 SNPs in six genomic regions totaling 2.5 Mb typed in all six populations. Three different tagSNP selection algorithms (based on haplotype diversity, haplotype entropy and linkage disequilibrium) were compared with each other and to see how well tagSNPs derived from one population sample would apply to other populations. For each algorithm, the resulting tagSNP sets from different populations were compared through the similarity measure (Liu et al. 2004, Genetic Epidemiology 27). The haplotype diversity based algorithm is most cost effective in tagSNP selection by preserving tagSNP transferability among regional populations with the minimum number of tagSNPs. However, it is dependent on block structure, an overly simplistic view of LD. Indeed, block patterns differ significantly among all populations, both within and between regions. However, the tagSNP sets show good transferability among populations within regions. The best tagSNP transferability among regional populations is achieved from pairwise LD tagging algorithm at a cost of 30~60% more tagSNPs per gene, and it is independent of block definition. In those instances in which Yoruba or Hausa tagSNPs did not suffice for African Americans (the diversity and LD based algorithms for two of the genomic regions) the union of tagSNPs from an African and European population included most of the tagSNPs selected directly for African Americans. We conclude that tagSNPs are usually generalizable within geographic regions but not exactly so. Supported in part by NIH GM57672.

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Positive Selection of a Pre-expansion CAG Repeat of the Human SCA2 Gene. *F. Yu[1], P. Sabeti[2], P. Hardenbol[3], Q. Fu[1], B. Fry[2], X. Lu[1], S. Ghose[1], R. Vega[1], A. Perez[1], S. Pasternak[1], S. Leal[1], T. Willis[3], D. Nelson[1], J. Belmont[1], R. Gibbs[1].* 1) Baylor College of Medicine, Human Genome Sequencing Center, 1 Baylor Plaza, Houston, TX, 77030, USA; 2) Broad Institute of MIT and Harvard, 1 Kendall Square, Cambridge, MA 02319, USA; 3) ParAllele Bioscience, Inc., 7300 Shoreline Court, South San Francisco, CA, 94080, USA.

A region of approximately one megabase of human Chromosome 12 shows extensive linkage disequilibrium (LD) in Americans with ancestry from northern and western Europe. This strikingly large LD block was analyzed with statistical and experimental methods to determine whether natural selection could be implicated in shaping the current genome structure. Extended Haplotype Homozygosity and Relative Extended Haplotype Homozygosity (EHH/REHH) analyses on this region mapped a core region of the strongest conserved haplotype to the exon 1 of the Spinocerebellar ataxia type 2 gene (*SCA2*, locusID: 6311). Direct DNA sequencing of this region of the *SCA2* gene revealed a significant association between a pre-expanded allele ($(CAG)_8CAA(CAG)_4CAA(CAG)_8$) of CAG repeats within exon 1 and the selected haplotype of the SCA2 gene. A significantly negative Tajima's D value (-2.20, $P < 0.01$) on this site consistently suggested selection on the CAG repeat. This region was also interrogated in the three other populations, none of which showed signs of selection. These results suggest that a recent positive selection of the pre-expansion *SCA2* CAG repeat has occurred in Europeans.

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Further evidence for a correlation of variation of CYP3A5*3 and AGT-M235T allele frequencies with distance from the equator: inclusion of Asian Indian populations. *T.J. Pemberton[1], D. Witonsky[2], C. Gonzales[1], J.W. Belmont[3], A. Di Rienzo[2], P.I. Patel[1].* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Department of Genetics, University of Chicago, Chicago, IL; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Large inter-ethnic variation in prevalence is known for a number of common conditions, such as hypertension. This variation may be due in part to large differences in risk allele frequencies between human populations which result either from the history of population structure and migration or from the action of natural selection on the risk variants themselves. Because selective pressures may vary with geography and other environmental variables, detectable patterns of geographic variation may be observed. Recently, allele frequencies at two single nucleotide polymorphisms within the CYP3A5 and AGT loci that have been shown to influence salt homeostasis were reported to correlate with distance from the equator (Thompson et al. AJHG 75:1059, 2004). Asian Indian populations have been largely excluded from the majority of large scale studies on genetic variation in populations, primarily because the Human Genome Diversity Panel does not include Asian Indian natives. This is despite their displaying a high prevalence of diseases linked to changes in diet and environment that have arisen as their lifestyle has become more westernised. The high prevalence of endogamy and relatively low admixture present in the population distinguishes Indian populations from most other populations presently used in population genetic studies. We have analysed these SNPs within a large Indian cohort of 577 individuals speaking 15 different Indian languages to determine the relative correlation in these populations with that of previously studied populations. Our data shows that these SNPs exhibit the same trend within our Indian population and that their inclusion in the analysis of the global population improves the correlation of the data. These results support the inclusion of an Indian population in future analysis of global population genetics.

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Detecting population stratification using 100K Affymetirx Mapping Array. *X. Mao[1], I. Halder[1], M. Bauchet[1], R. Mei[2], C. Shweta[4], E. Burchard[4], E. Ziv[4], M. Shriver[4], N. Schork[3].* 1) Department of Anthropology, Penn State University, State College, PA; 2) Affymetrix, Inc., 3380 Central Expressway, Santa Clara, California 95051, USA; 3) Department of Psychiatry, University of California, San Diego, CA; 4) Dartment of Medicine, University of California, San Francisco.

DNA microarray platforms have increased the efficiency of biomedical research substantially. Likewise, greater analytical effect is required to summarize the information and interpret the results. We have typed the 100K Affymetrix Mapping Array on 267 individuals from five major groups (85 African Americans and West Africans, 115 European Americans, 17 South East Asians, 20 East Asians and 30 Indigenous Americans) and use the resulting multilocus genotypes to explore two methods of detecting population structure, a principle coordinate-based method and a pairwise distance-based method. Correlation of principle coordinates estimates from unlinked subsets of SNP markers is one way to explore the pattern of population structure. Populations with higher correlations have more stratification while populations with insignificant correlations have no stratification. In our analysis, African Americans and European Americans show the highest levels of correlation indication, while the East Asian group shows the smallest amount of correlation. The pairwise allele sharing distance estimates of individuals is an analogous approach to study substructure. Correlations of pairwise distance measure from unlinked subsets of markers (even vs. odd chromosomes) not only have the same trend as principle coordinates correlations, but also can be used to pinpoint the causation of population heterogeneity to certain individuals. Importantly, we can test for correlations between locus specific pairwise distance and overall pairwise distance measured across the genome as a method of quantifying the informativeness of previously unselected markers to facilitate the identification of ancestry informative markers.

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Population differentiation of a SE Asian isolate from major Asian populations using 36 microsatellites. *J. Listman[1], R.T. Mailson[2,3], A. Sughondhabirom[4], N. Thavichachart[4], S. Tangwonchai[4], A. Mutirangura[4], T.R. Disotell[1], J. Gelernter[2,3].* 1) Dept Anthropology, New York Univ, New York, NY; 2) Dept Psychiatry, Yale Univ Sch Medicine, New Haven, CT; 3) VA CT, West Haven, CT; 4) Chulalongkorn Faculty of Med, Bangkok, Thailand.

The ability to differentiate between populations is valuable for admixture detection in case-control association studies and for inferring interpopulation relationships in anthropological studies. This is easy to achieve for major continental populations. We investigated whether this could be accomplished with more closely related populations. Unrelated samples of self-identified Thai (N=53) and Chinese (N=37) obtained in Bangkok, Thailand and Hmong (N=45) obtained from a village in northern Thailand were genotyped with 36 unlinked autosomal microsatellite markers. These markers were previously demonstrated to collectively differentiate between African Americans and European Americans. The mean delta for this panel was 0.25 (between Hmong and Chinese), 0.24 (between Hmong and Thai), and 0.17 (between Chinese and Thai). The program, STRUCTURE, which implements Bayesian clustering was used to assess the ability of this marker set to differentiate between the three Asian populations. The program MER was used to detect and remove any related individuals who may not have identified themselves as such. The Hmong were allocated into a distinct cluster with 91.4% estimated ancestry (K=2). Thus, we were able to differentiate an Asian tribal population from larger Asian populations. It was not possible to differentiate between the Thai and Chinese with this marker set irrespective of the presence of the Hmong in the analysis. Success in differentiating within these continental populations demonstrates that population structure is detectable at this level. This could be due to genetic drift shaping the gene pool of an endogamous population or the effects of sampling Hmong from a village in contrast to Thai and Chinese from an urban population. The results also suggest that superficial phenotype (i.e., continental origin) may mask population history and support the possibility of admixture mapping in closely-related populations.

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Founder effects and diversity for hereditary eye diseases in Newfoundland and Labrador. *J. Green[1,2,3], J. Whelan[2], D. Bautista[2], S. Moore[3], K. Grewal[1], M. O'Driscoll[1], B. Younghusband[1], P. Parfrey[3], S. Murphy[3].* 1) Disc of Genetics; 2) Disc of Ophthalmology; 3) Disc of Medicine, Memorial Univ, St. John's, NL, Canada.

PURPOSE: Newfoundland and Labrador (NL) has been described as a genetic isolate with founder effect and increased prevalence of certain hereditary diseases. Ancestors were English (protestant) or Irish (catholic). Settlements were coastal remaining isolated until recently, and family size was large. Both geographic and religious isolation occurred. We were interested in the proportion of blindness in NL attributable to hereditary eye disorders, and in the genetic epidemiology of hereditary eye disease. **METHOD:** Records of the Canadian National Institute of the Blind (CNIB) were reviewed, patients seen in local and outreach clinics, and affected and unaffected members of families with unusually frequent conditions were recruited and consented for clinical and molecular genetic analysis. **RESULTS:** At least 24% of CNIB registrants had a single gene disorder as a cause of blindness including retinal dystrophies, ocular and oculocutaneous albinism, and anterior segment anomalies. A cluster of patients with a distinctive early-onset retinal dystrophy (Newfoundland Rod-Cone Dystrophy (NFRCD)) was identified. Families with NFRCD live within a 10 mile radius. Surprisingly there were two different mutations of the *RLBP1* gene in these 27 individuals from one genetic isolate. Bardet-Biedl Syndrome (BBS), a complex condition involving a severe retinal dystrophy, obesity, polydactyly, learning difficulties, renal and endocrine abnormalities, is unusually prevalent. Although one founder mutation was expected, seven different mutations in five BBS genes have been identified. There is as much intra-familial as interfamilial variability in these families, and no genotype/phenotype correlation is apparent. **CONCLUSIONS:** NL has multiple genetic isolates rather than being one homogenous genetic isolate. This knowledge is important as current and future studies focus on blindness caused by complex traits including high myopia, cataracts, glaucoma, and age-related macular degeneration.

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Variation of individual admixture within and between populations follows continuous distributions. *I. Halder[1], C. Nievergelt[2], R. Ferrell[3], C. Gu[4], D. Arnett[5], S. Kardia[6], R. Cooper[7], X. Zhu[7], N. Risch[8], C. Hanis[9], N. Schork[2], M.D. Shriver[1].* 1) Pennsylvania State University; 2) University of California, San Diego; 3) University of Pittsburgh; 4) Washington University; 5) University of Alabama; 6) University of Michigan; 7) Loyola University; 8) University of California, San Francisco; 9) University of Texas, Houston.

Admixture stratification is a well documented cause of confounding in association studies. We have estimated individual ancestry and tested for presence of admixture stratification in the populations that are part of the Family Blood Pressure Program. Individuals studied are from several African-American (AA) and European-American (EA) populations and one Mexican-American (MA) population. All individuals were typed for a common panel of 311 microsatellite markers. Individual ancestry estimated using Maximum Likelihood and a separate Bayesian method implemented in the program STRUCTURE, show a continuum of ancestry proportions with substantial variation within different self identified race/ethnicity groups and some degree of overlap between two of the groups. We have tested for and observed admixture stratification in all of the AA and MA samples, but not in any of the EA samples. We have additionally observed correlations between admixture proportions and some phenotypes. We conclude that since categorical descriptions of individuals are non-overlapping and do not describe within group variation, they are suboptimal for describing human genetic variation within and among populations and metapopulations. Continuous expressions of genetic variation such as individual admixture provide more detailed descriptions of the genetic structure of a population.

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Characterization of a novel private allele for East Asia and Oceania. *J.L. Peterson[1], J.E. Ekins[1], K.L. Pennington[1], K.H. Ritchie[1], L.A.D. Hutchison[1], N.M. Myres[1], J.B. Ekins[1], J.E. Gomez[1], A.A. Nelson[1], M.E. Nelson[1], L. Layton[1], M.L. Lunt[1], T.A. Tolley[1], S.S. Masek[1], U.A. Perego[1], S.R. Woodward[1,2].* 1) Sorenson Molecular Genealogy Foundation (SMGF), Salt Lake City, UT, USA; 2) Dept. of Microbiology, Brigham Young University, Provo, UT, USA.

Genetic data as well as anthropological and linguistic evidence suggest that modern inhabitants of Oceania could have originated in a variety of world locations, including Asia. A large amount of the genetic data studied is based on either Y chromosomal or mitochondrial analysis (e.g. the Polynesian Motif), however, few of these studies have incorporated the X-chromosome (X-chr) as evidence of population origins. Herein we characterize a novel private allele on the X chr for the Oceanic region and East Asia. We have identified a 1-bp mutation in the STR locus DXS1041. 176 of 18,065 samples genotyped at that locus have this mutation, giving a frequency of 0.974% in the SMGF database. Of these samples, 172 have X line genealogies deeply rooted in Asia or Oceania: 66.9% of these are Polynesian, 22.1% Asian, 4.1% of New Zealand origin, 2.3% Filipino, 2.3% Melanesian, and 2.3% are mixed from both Asian and Oceanic populations. Herein we propose that this allele could be used to identify membership in Asian/Oceanic populations.

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Population bottlenecks and neutral evolution as major shaping forces of human genome architecture. *N. Katsanis[1], P.E. Chen[1], T.M. Teslovich[1], P. Stankiewicz[2], C.S. Kashuk[1], S.P. Zack[3], J.M. Organ[3], M. Withers[2], A. Chakravarti[1], J.R. Lupski[2], D.J. Cutler[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Center for Functional Anatomy and Evolution, Johns Hopkins University, Baltimore, MD.

The neo-Darwinian view of human evolution proposes that the fixation of novel mutations is driven by the selective advantage they proffer to reproductive fitness. When considering the global architecture of the human genome, a similar model has been evoked to explain the rapid acquisition and proliferation of exogenous DNA. To understand the evolutionary forces that have morphed human genome architecture, we investigated the origin, composition, and functional potential of *numts* (nuclear mitochondrial pseudogenes), partial copies of the mitochondrial genome found abundantly in chromosomal DNA. Our data indicate that these elements are likely to be evolutionarily neutral or deleterious, since they possess no gross positional, transcriptional, or translational features that might indicate functionality subsequent to integration. Using sequence analysis and fossil data, we also show a burst of integration of *numts* in the primate lineage that centers on the prosimian-simian split and mimics closely the temporal distribution of Alu and processed pseudogene acquisition. We suggest that the gross architecture and repeat distribution of the human genome can be largely accounted for by a population bottleneck early in the simian lineage and subsequent neutral fixation of repetitive DNA, rather than positive selection or unusual insertion pressures.

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Haplotype diversity and population histories in the CEPH Human Genome Diversity Panel based upon variation in four cytochrome P450 genes and COMT. *W. Modi[1], H. Sicotte[1], D. Garcia-Ross[1], M. Yeager-Jeffery[1], R. Welch[1], L. Burdette[1], H. Cann[2], G. Thomas[2], S. Chanock[3].* 1) SAIC, NCI-ATC, Bethesda, MD; 2) CEPH, Paris, France; 3) NCI-Core Genotyping Facility, Bethesda, MD 20892.

Central to the mission of ascertaining the importance of genetic variation in human health and disease is understanding patterns of variation in different populations throughout the world. Assessing geographic variation enables us to evaluate the relative contributions of selection, drift and admixture in molding the architecture of the human genome. This study is estimating nucleotide and haplotype diversity, times to most recent common ancestory through coalescent simulations, and migration patterns among nearly 1500 individuals belonging to over 55 populations from the CEPH Human Genome Diversity Panel, and the HapMap and SNP500 reference populations, based upon variation in cytochrome P450 genes and COMT. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Catechol-O-methyltransferase (COMT) catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. Data collection includes: 1) the resequencing of all coding and adjacent flanking regions in 102 individuals assigned to four ethnic groups. This has identified about 300 SNPs from 60 kb of sequence revealing a frequency of one SNP every 200 bp. 2) Genotyping common SNPs every 2 kb throughout each gene in 1500 individuals: CYP1A1 and CYP1A2 (22 SNPs covering 62 kb), CYP1B1 (18 SNPs covering 22 kb), CYP3A4 (18 SNPs covering 49 kb) and COMT (31 SNPs covering 59 kb). Preliminary results indicate extensive intergenic variability in nucelotide diversity and interpopulation variation in allele and haplotype frequencies suggesting varied selective pressures and/or population histories.

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Single molecule recovery of human L1 insertions from sperm DNA. *R.M. Badge[1], A.J. Jeffreys[1], J.V. Moran[2].* 1) Dept Genetics, Univ Leicester, Leicester, United Kingdom; 2) Dept Human Genetics, Univ Michigan Medical School, Ann Arbor, Michigan, USA.

L1 is our most successful and currently only autonomous transposable element. As such it contributes to mutational load directly through insertional mutagenesis and indirectly through ectopic recombination, and likely mobilises all other dispersed repeats currently remodelling the human genome. Thus L1 drives genome fluidity in humans, but the rate at which this process occurs in germline cells is not known. Cell culture retrotransposition assays have revealed our genome contains highly active elements that show presence/absence polymorphism. These assays are also powerful tools to study the mechanism of L1 retrotransposition but cannot estimate the rate of retrotransposition of endogenous human elements. ATLAS (Badge *et al*, 2003) is a genomic display technique that can selectively display full-length L1 terminus/genomic DNA junctions from the most active L1 subfamilies. Simultaneous display of full-length L1s from many human genomes makes the isolation of low allele frequency, and thus likely young and still active elements, relatively trivial. In addition the application of ATLAS to very small numbers (pools) of sperm genomes can potentially enable the display of *de novo* insertions by gel electrophoresis, despite their origin as unique L1/genomic junctions within individual sperm genomes. Here we describe the development of an ATLAS variant, Small Pool (SP) ATLAS that can demonstrably recover molecularly unique events from human sperm DNA and that shows levels of reproducibility and specificity that are compatible with the recovery of *de novo* L1 insertions. Dilution of display inputs to the single molecule level allows estimation amount of DNA scanned per reaction, thus placing an upper limit on the rate. Importantly these rates are specific for insertions of intact L1s and thus enable us to quantify the most relevant evolutionary parameter- the rate at which retrotransposition competant elements are inserted into our genome. Badge RM, Alisch RS, Moran JV. ATLAS: a system to selectively identify human-specific L1 insertions. Am J Hum Genet. 2003 Apr;72(4):823-38.

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Hybridisation enrichment recovery of *de novo* human L1 insertions from sperm DNA. *P.J. Freeman, R.M. Badge, A.J. Jeffreys.* Dept Genetics, Univ Leicester, Leicester, UK.

The most important class of mobile DNA in humans is L1, an autonomous non-LTR retrotransposon. L1 retrotransposition has contributed to the remodeling of the human genome through insertional mutagenesis, transduction, pseudogene formation, and ectopic recombination. There is strong evidence that L1s have played an important role in changing human DNA, and also that they are still doing it today. However L1 retrotransposition dynamics are little understood in the human germline due to the lack of stable human germline cell culture techniques and the fact that *de novo* insertions occur at approximately one event genome-wide per 50 - 100 haploid genomes. Hybridisation enrichment can be used to directly access *de novo* L1 insertions in human sperm genomic DNA, from targeted loci. L1 specific biotinylated oligonucleotides (bio-oligos) are used to enrich specific DNA sequences that are under-represented in an aliquot of DNA. Enrichment is achieved by binding bio-oligos to the target sequence and physical retrieval of the bio-oligo bound single stranded DNA using streptavidin-coated paramagnetic beads. Successive rounds of PCR and enrichment can be used to increase yield. The use of the bio-oligos directly on sperm DNA would be futile due to the amount of L1 sequence dispersed throughout the genome. In order to detect *de novo* insertions in sperm DNA, the search area has to be limited to genomic sections (~ 5kb in length) that are devoid of L1 sequences. An important technical challenge is that the PCR amplification of *de novo* insertions (up to 12kb in length) has to be efficient at the single molecule level. A *de novo* insertion will appear once in a pool of sperm DNA, and thus must be amplifiable even in the presence of an overwhelming majority of empty target sites. Also to screen sufficient DNA to be sure to recover many *de novo* L1 insertions multiple target loci must be simultaneously amplified requiring long-range multiplex PCR. Here we demonstrate that these technical challenges have been overcome and in principle that even at the lowest estimated rate of insertion (1 insertion in 2 X 10^8 kb) at least 10 *de novo* insertions should be recovered per ejaculate.

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Substantial variation in retrotransposition potential generated by many alleles of young human L1 retrotransposons. *M.dC. Seleme, M.R. Vetter, H.H. Kazazian.* Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Even though they are rare in the human genome(0.02%), young active L1 retrotransposons can generate large variation among individuals through various molecular events such as insertional mutagenesis, exon shuffling, and mediating the dispersion of processed pseudogenes, Alu, and SVA elements. Here, in four populations (160 genomes) we have investigated a type of variation generated by active L1s that is based on the natural substitutions that occurred along their full-length sequence after insertion. Interestingly, we have found a large number of alleles at each of 3 L1 loci analyzed. Roughly 1 new allele was found for every 3 full-length sequences analyzed at each locus. For example, among 73 genomes containing a particular L1, 25 alleles were identified. Among the various nucleotide changes, we found nonsense mutations, insertions and deletions. Assuming a neutral mutation rate, the polymorphism data suggested that the insertions occurred from about 356.000 years ago for the oldest to about 80.000 years ago for the youngest. Along with the allelic description of these three L1 loci, we studied the allelic retrotransposition capacity ex-vivo. We grouped the alleles according to their average retrotransposition capacity and found that many single or combined nucleotide changes dramatically affected the retrotransposition efficiency of the elements, generating an unsuspected high degree of individual variation in retrotransposition potential. For instance, one element presented variants with activity as low as 5% and as high as 180% as compared to a reference element; another element presented inactive alleles and alleles with activity as high as 100%. This is the first large-scale analysis on the polymorphism of single, potentially active L1s at specific genomic locations and provides new insights into 1) how quickly recently inserted L1s can become inactivated by mutation and 2) the extent of individual variation in retrotransposition capability.

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Identification of proteins that bind galectin-2, encoded by a gene associated with myocardial infarction using tandem affinity purification method. *K. Ozaki[1], K. Inoue[2], K. Odashiro[2], M. Nobuyoshi[2], Y. Nakamura[1,3], T. Tanaka[1].* 1) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 2) Department of Cardiology, Kokura Memorial Hospital, Kitakyushu, Japan; 3) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Myocardial infarction might result from the interactions of multiple genetic and environmental factors, none of which can cause disease solely by each of themselves. We have previously identified galectin-2 protein as a binding partner of lymphotoxin- (LTA) whose functional single nucleotide polymorphisms (SNPs) conferred susceptibility to myocardial infarction. The association study further revealed that a functional SNP in *LGALS2* encoding galectin-2, which led to altered secretion of LTA, was also indicated a risk of myocardial infarction. To clarify detailed function of galectin-2 protein, we searched for its binding partners by using a tandem affinity purification method and identified several molecules. Toll like receptor 2 (TLR2) was one of them, which plays an important role in immune systems. Combined with previous results that LTA and tubulins also bind with galectin-2 and that tubulins have been reported to be used in the intracellular transport of membrane protein, we hypothesized galectin-2 plays a pivotal role in the transport of various membrane/secreted protein that constitute immune system including inflammation.

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A novel DFNA9 mutation in the vWFA2 domain of COCH alters a conserved cysteine residue and intrachain disulfide bond formation resulting in progressive hearing loss and site-specific vestibular and central oculomotor dysfunction. *V.A. Street[1], J.C. Kallman[1], N.G. Robertson[2], S.F. Kuo[2,5], C.C. Morton[2,3,4], J.O. Phillips[1].* 1) V.M. Bloedel Hearing Research Ctr, Otolaryngology - HNS Department, University of Washington, Seattle, WA; 2) Department of Obstetrics, Gynecology and Reproductive Biology; 3) Department of Pathology, Brigham and Women's Hospital; 4) Harvard Medical School, Boston, MA; 5) Speech and Hearing Bioscience and Technology Program, Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

Statement of Purpose: Hearing and balance impairment are common clinical complaints. However, the number of single gene mutations known to precipitate both sensory problems in one individual or family is small. It is unclear whether auditory and vestibular dysfunction generally represent separate entities or whether the presence of vestibular symptoms is not fully appreciated without formal vestibular assessment in human pedigrees segregating hearing loss. In this abstract, we report the characterization of a large American pedigree (referred to as the HL3 family) that self-reports inherited hearing loss. Methods Used: Audiologic evaluations consisted of immittance testing and establishment of pure-tone air and bone conduction thresholds. Vestibular assessment included oculomotor testing, computerized dynamic posturography, rotational testing, vestibular evoked myogenic potentials, and caloric testing. Genetic linkage, haplotype analysis, and mutation scanning was performed on DNA samples from HL3 individuals. Transient transfections and Western blot analysis were used to characterize the identified mutation. Summary of Results: Autosomal dominant hearing loss in the HL3 pedigree was mapped genetically to the long arm of chromosome 14 in band q12. A maximal pairwise LOD score of 7.08 was obtained with marker D14S1021. A mutation within exon 12 of the COCH gene (encoding the cochlin protein) was identified in affected HL3 family members. The mutation results in a predicted substitution at an evolutionarily conserved cysteine residue in the C-terminus of cochlin. This COCH exon 12 alteration represents the first reported mutation outside of the cochlin LCCL domain which is encoded by exons 4 and 5. The exon 12 mutant cochlin is translated and secreted by transfected mammalian cells. Western blot analysis under non-reducing and reducing conditions suggests that this exon 12 mutation alters intramolecular cochlin disulfide bond formation. In the vestibular system, a progressive horizontal canal hypofunction and a saccular otolith crisis were detected in HL3 family members with the COCH alteration. Abnormal central oculomotor test results in HL3 family members with the COCH alteration imply a possible central nervous system change.

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Auditory and vision phenotype of waltzer *Cdh23$^{v\text{-}6J}$* and Ames waltzer *Pcdh15$^{av\text{-}Jfb}$* double mutant mice: no evidence of retinitis pigmentosa or digenic inheritance of progressive hearing loss. *Z. Ahmed[1], R.J.L. Haywood-Watson II[1,2], S. Riazuddin[1], S. Kjellstrom[1], R.A. Bush[1], L.L. Hampton[3], J.F. Battey[1,3], P.A. Sieving[1,4], G. Frolenkov[1], T.B. Friedman[1].* 1) Lab Molecular Genetics, NIDCD, National Institutes of Health, Rockville, MD; 2) Molecular and Cellular Biology Program, Tulane University, New Orleans, LA; 3) National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 4) National Eye Institute, National Institutes of Health, Bethesda, MD.

Mutations of *CDH23* or *PCDH15* are associated with Usher syndrome type I. Recently, a digenic interaction between these two genes was reported to be responsible for progressive hearing loss in doubly heterozygous mice (+ *Cdh23$^{v\text{-}2J}$* / *Pcdh15$^{av\text{-}3J}$* +) and for Usher syndrome type I in humans (Zheng et al. 2005). Here, we provide evidence that mice (n = 44) which are double heterozygotes for both *Cdh23$^{v\text{-}6J}$* and *Pcdh15$^{av\text{-}Jfb}$* alleles in cis or in trans on a uniform C57BL/6J background do not exhibit progressive hearing impairment beyond the age related hearing loss seen in control mice (n = 51). No stereocilia defects were observed in P2 *Cdh23$^{v\text{-}6J}$ Pcdh15$^{av\text{-}Jfb}$* double heterozygotes (P120) and neither the *Cdh23$^{v\text{-}6J}$* nor *Pcdh15$^{av\text{-}Jfb}$* homozygotes were more affected by the presence of one mutant copy of *Pcdh15$^{av\text{-}Jfb}$* or *Cdh23$^{v\text{-}6J}$*, respectively. We also document the auditory and visual phenotype of mice that are double homozygotes for *Cdh23$^{v\text{-}6J}$* and *Pcdh15$^{av\text{-}Jfb}$* mutations. Although, there is no retinal abnormality detected in the double homozygous mutant mice, scanning electron microscopic studies revealed, as expected, prominent abnormalities of inner ear hair cell stereocilia bundles.

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State Space Methods for Studying Dynamic Behavior of Gene Regulation. *X. Sun[1], L. Jin[1], J. Qian[1], M. Xiong[2].* 1) Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas Health Scince Center.

Gene regulation is a complex system that requires accomplishing complex tasks with high accuracy. A very powerful approach to modeling complex systems is the state-space approach. Mechanisms for using RNA interference and artificial genetic networks as target gene therapy are systematical control of the state of the cell to minimize malfunctions of the cells. Their successful applications to the development of smart drugs require state-space representation of dynamics of gene regulation and genetic networks and applications of modern control theory to design and analysis of regulatory systems. State-space approach provides a general framework and a powerful tool for application of control theory to analysis of gene regulation. The purpose of this report is to develop state-space models for gene regulation, study transient response of gene expression under environment stimuli and classify genes according to their transient response to external perturbation. To achieve this goal, State-space representation of regulation of a gene and study second order systems for gene regulation will be presented. Interpretation of coefficients in the second order systems as resistance, capacitance and inductance will be studied. Mathematical methods for transient response analysis of gene regulation will be developed. The proposed methods will be applied to cell cycle of yeast gene expression data. Classification of genes in terms of their transient response behavior for the yeast gene expression data set will be performed.

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Idenfitication of ABCA4 regulatory mutations in recessive Stargart families. *C.M. Zaremba[1], W. Wiszniewski[1], R.A. Lewis[1,2,3,4], E.H. Marguiles[5], E.D. Green[5], J.R. Lupski[1,2].* 1) Department of Molecular and Human Genetics; 2) Department of Pediatrics; 3) Department of Medicine; 4) Department of Ophthalmology; 5) Genome Technology Branch and NISC, National Human Genome Research Institute.

Stargart disease (STGD1) is an autosomal recessive retinal dystrophy caused by alterations in *ABCA4*. Mutational analysis of recessive STGD has been limited to direct DNA sequencing of the exon coding regions, in which 66-80% of mutant alleles have been identified; thus leaving a significant fraction of mutant alleles unaccounted for. In an effort to identify the remainder of the causative alterations, we employed a method to elucidate highly conserved sequences by comparative sequence analysis in a 300-Kb region surrounding *ABCA4* followed by direct DNA sequencing and functional analysis of these conserved fragments. Initial prediction of potential functional elements in the 5 UTR of *ABCA4* was implemented using a comparative genomic strategy across eight vertebrate species (human, chimpanzee, armadillo, cat, dog, mouse, pig, and opossum). Using this approach we identified 32 conserved fragments (39.59 bp average) in a 10-Kb interval just upstream of the 5 UTR of *ABCA4*. Furthermore, we screened for alterations in these conserved sequences in two groups of STGD families with multiple (greater than two) affected siblings possessing either zero (16 families) or one (40 families) *ABCA4* coding region alterations. Presently, we have identified four potentiialy significant SNPs: -1439 A>T (12.0%), -1086 A>C (29.2%), -900 A>T (34.9%), and -761 C>A (1.7%). Ongoing experiments are addressing whether these represent functional SNPs. Collectively, these data support the contention that a fraction of mutations are undetected in patients with recessive STGD and may reside in the non-coding, potentially regulatory regions of *ABCA4*.

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Evaluating dependency among three systems: analysis of the relationships between a pair of transcriptional regulatory elements and tissue specific gene expression using the double and triple mutual information in humans. *M. Tanino[1,2], M. Watanabe[3], Y. Takahashi[3], T. Tamura[4], T. Imanishi[2], T. Gojobori[2,5].* 1) Integrated Database Group, JBIRC, JBIC, Tokyo, Japan,; 2) Integrated Database Group, BIRC, AIST, Tokyo, Japan; 3) Fujitsu Co. Ltd, Tokyo, Japan; 4) BITS Co., Ltd, Shizuoka, Japan; 5) Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Shizuoka, Japan.

To date, there have been many reports investigating the relationship between transcriptional factors and their tissue-specific regulatory mechanisms. However, few reports have discussed or evaluated the combinatorial effects of transcriptional factors on regulatory mechanisms. Transcriptional regulation, especially in multi-cellular eukaryotes, is a complex system in which different types of transcriptional factors work together through DNA binding sites (cis-acting elements). Thus, the analysis of the relationship between a pair of cis-acting elements in the upstream regions of genes and the expression patterns must be effective for understanding the transcriptional regulatory mechanism in multi-cellular eukaryotes. The double (usual) mutual information gives a general measure of correlation between two systems and this index is useful as long as we consider only two systems. However, in the case of three systems, even when two systems seem to be independent, for example the relationship between a cis-acting element and expression pattern, if we consider another cis-acting element together with these, we may observe a mutual dependence among these three systems. We present here a method of evaluating inter dependency among three systems, a pair of cis-acting elements and an expression pattern, using the double and triple mutual information. Our method should be very useful in unraveling the transcriptional regulatory mechanism where not a single factor but multiple factors cooperate to govern the mechanism. We scored all pairs of cis-acting elements stored in the TRANSFAC database and found several novel pairs of cis-acting elements which may regulate tissue-specific expression under normal conditions in adult humans.

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In Vivo Functional Study of a Unique Polyglutamine Stretch in RUNX2. *P. Fonseca[1], G. Zhou[1], Y. Chen[1], B. Lee[1,2].* 1) Dept Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

RUNX2 is a non-redundant transcriptional activator involved in osteoblast differentiation and chondrocyte maturation. It contains a unique N-terminal polyglutamine-alanine (Q/A) domain consisting of a stretch of 23 residues of glutamine and 17 residues of alanine. Mutations in RUNX2 cause an inherited skeletal malformation syndrome, cleidocranial dysplasia (CCD). Until now, only one expansion in the alanine tract has been reported in an individual with CCD, while contractions of the tract are benign polymorphic variants. Polyglutamine expansions are known to cause several neurodegenerative diseases, but the function of the QA tract in RUNX2 is still unclear. In this study, we generated a series of mutant forms of RUNX2 with a deleted (7QE2A-RUNX2) or expanded (72QE23A-RUNX2) QA domain. In vitro studies show that polyglutamine deletion (7QE2A-RUNX2) decreases transactivation of the osteocalcin reporter, while expansions only reduce transactivation when they exceed 70 repeats. Mice over expressing the deletion mutation 7QE2A-RUNX2 under the osteoblast-specific Col1a1 promoter present with short stature at birth. Skeletal preparations revealed delayed closure of fontanel while clavicles appeared normal. Radiographic analysis of transgenic mice suggests lower bone mass. Transgenic mice also show absence of upper teeth and overgrown lower teeth with malocclusion suggesting this mutant was able to interfere with RUNX2 function during intramembranous ossification thereby phenocopying CCD in the skull. Interestingly, a fragment containing only the QA domain exhibited a stronger dominant-negative effect on full-length RUNX2 in RUNX2 transactivation assay in ROS17 cells. In vivo, transgenic mice over expressing only the QA domain in osteoblasts exhibited dwarfism and lower bone density. These data suggest that over expression of normal length Q/A domain may have dominant negative effects on osteoblast function independent of the RUNT domain. The analysis of these different transgenic mice will lead to a better understanding of the function of the Q/A tract in RUNX2 and potentially identify novel human phenotypes that might be allelic to CCD.

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Two novel point mutations in the long-range Shh enhancer in three families with triphalangeal thumb and preaxial polydactyly. *C.A. Gurnett[1], A.M. Bowcock[2], F.R. Dietz[3], J.A. Morcuende[3], J.C. Murray[4], M.B. Dobbs[5].* 1) Dept Neurology, Washington Univ, St Louis, MO; 2) Dept Genetics, Washington Univ, St Louis, MO; 3) Dept Orthopaedics, University of Iowa Carver College of Medicine, Iowa City, IA; 4) Dept Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA; 5) Dept Orthopaedics, Washington Univ, St Louis, MO.

Spatio-temporal expression of Shh is driven by a regulatory element (ZRS) that lies 1 Mb upstream from Shh. Point mutations within the highly conserved ZRS have been described in the hemimelic extra toes mouse and in four families with preaxial polydactyly (Lettice et al., 2003). Four North American Caucasian families were identified with autosomal dominant triphalangeal thumb. DNA from 20 affected and 36 unaffected family members was evaluated by sequence analysis of a 774 bp highly conserved ZRS contained within Lmbr1 intron 5. Mutations within ZRS were identified in three of four families. In pedigree A and C, a novel A/G transition was identified near the 5' end of ZRS at bp 739 that segregated with disease or carrier status. Pedigree A, described previously (Dobbs et al., 2000), is a large family with 19 affected members who exhibit a milder phenotype, including predominantly triphalangeal thumbs and low penetrance relative to other families (82%). Pedigree C is a small family with two affected family members with triphalangeal thumb, and one affected with both triphalangeal thumb and preaxial polydactyly. A novel C/G mutation at bp 621 was identified in pedigree B that segregated with the disease in all four affected individuals who manifested both preaxial polydactyly and triphalangeal thumb. Mutations within ZRS appear to be a common cause of familial triphalangeal thumb and preaxial polydactyly. A genotype/phenotype correlate is suggested by pedigree A, whose mutation lies near the 5' end of ZRS; this family demonstrates a higher rate of nonpenetrance and milder phenotype. However, modifier genes may be contributing to the milder phenotype in this family.

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Expression of sarcoglycans in brain reveals potential role of -SG in myoclonus dystonia. *H. Cai, L. Zweier, W. Shi, J. Chen, Y.M. Chan.* The Henry Hood Research Program, Sigfried and Janet Weis Center for Research, the Geisinger Clinic, Danville, PA 17822.

Sarcoglycans are a group of transmembrane proteins predominantly expressed in skeletal and cardiac muscle. Currently, six sarcoglycans have been identified (,,,,,-SG). In muscle, , , , and -SG form a tetra-meric complex on the sarcolemma. The proper assembly and targeting of sarcoglycans to the plasma membrane are dependent on the formation of a central /-SG core. Mutations in muscle sarcoglycans have been shown to cause autosomal-recessive limb-girdle muscular dystrophies (LGMD) and dilated cardiomyopathy. On the other hand, mutations in -SG are responsible for a movement disorder called myoclonus dystonia. In this study, we compared the expression of sarcoglycans in brain with other tissues. Previously, we have demonstrated that sarcoglycans form a distinct complex in Schwann cells consisting of -SG, -SG, -SG and -SG. They are localized to the outermost membrane of the Schwann cell and their expression correlates with peripheral nerve development. In this report, we provided evidence that and -SG are abundantly expressed in brain. However, -SG and -SG are barely detectable while -SG and -SG are not present in brain as demonstrated by both protein and RNA expression studies. Co-immunoprecipitation experiment indicated that -SG does not tightly associate with -SG. Our data suggested that -SG and -SG could have different functions in brain. Although no mutation has been associated with -SG, mutation screening in -SG should be included for myoclonus dystonia or other neurological disorders in the future.

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A Novel De novo Missense Mutation on the Grandpaternal X Chromosome in the JmjC Domain of the *JARID1C* Gene in an XLMR Family Linked to Xp11.3. *F.E. Abidi[1], L. Holloway[1], L.M. Wolf[2], R.E. Stevenson[1], C.E. Schwartz[1].* 1) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, South Carolina, USA; 2) Department of Pediatrics/ Human Development, Michigan State University, East Lansing, Michigan, USA.

Mutations in the *JARIDIC/SMCX* gene were recently found to cause X-linked mental retardation. As a part of our XLMR candidate gene testing, mutation analysis of the *JARIDIC* gene was done in the probands from 24 XLMR families linked to Xp11.2. We identified a novel missense mutation (p.V504M) in one of these families (K8835). The family consists of three affected males in two generations with mild - moderate MR. All three exhibited developmental delay as well as delayed speech and short stature (~10-15 % tile). The alteration, c.1510G>A, changes a conserved valine residue at position 504 to a methionine (p.V504M) and segregates with the phenotype. The analysis of markers flanking the *JARIDIC* gene indicates that this mutation is a de novo event arising on the grandpaternal X chromosome. The p.V504M mutation was not observed in 609 X chromosomes from normal individuals (239 males and 185 females) which would rule out the mutation being a rare polymorphism. This mutation falls in the JmjC domain of the *JARIDIC* gene where no previous mutations have been reported. Out of the 4 missense mutations previously reported only one was found to be present in the C5HC2 zinc finger domain, whereas the three other mutations were not located in any conserved domains of the gene. It has been postulated that the *JARIDIC* gene may have a role in histone modification because of the presence of the JmjC domain. Functional studies using the above mutation may help us understand the function of this domain in the *JARIDIC* gene. Our finding further confirms the hypothesis that this gene may play an important role in human brain function.

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Protein composition of the intranuclear inclusions of FXTAS. *P.J. Hagerman[1], C.K. Iwahashi[1], D.H. Yasui[1], H.J. An[2], C.M. Greco[3], F. Tassone[1], K. Nannen[1], B. Babineau[1], C.B. Lebrilla[2], R.J. Hagerman[4].* 1) Dept Biochem & Molec Med, UC Davis, Davis CA; 2) Dept Chem, UC Davis, Davis CA; 3) Dept of Path, UC Davis, Sacramento CA; 4) MIND Inst, UC Davis, Sacramento CA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder involving progressive intention tremor, gait ataxia, parkinsonism, and progressive memory loss. The selectivity of FXTAS for carriers of transcriptionally active, premutation alleles, coupled with the elevated levels of *FMR1* mRNA in the premutation range, led us to propose an RNA toxic gain-of-function model for FXTAS (Neurology 2001 57:127). The pathologic hallmark of FXTAS is the ubiquitin-positive intranuclear inclusion, found in neurons and astrocytes in broad distribution throughout the brain. A central prediction of the RNA toxicity model for FXTAS is that one or more RNA binding proteins is necessary to transduce the effect of the abnormal *FMR1* mRNA. Consistent with this hypothesis, we previously reported that the inclusions do contain *FMR1* mRNA.

Using a combination of nuclear isolation and preparative particle sorting protocols, we have now successfully isolated highly purified intranuclear inclusions from post-mortem brain tissue of FXTAS patients. Determination of the identities of the proteins comprising the inclusions has been greatly facilitated by the use of modern mass spectrometry approaches. Further analysis of the purified inclusions has enabled us to identify more than twenty proteins in the inclusions, including two RNA binding proteins, hnRNP A2 and MBNL1 (a homologue of *Drosophila* muscleblind), which are potential mediators of the RNA toxicity. FMRP itself appears to be absent from the inclusions. The inclusions also appear to contain several intermediate filament (IF) proteins, including lamins A/C, -internexin, and other neurofilament (NF) proteins. Although the role(s) of the A-type nuclear lamins is only beginning to emerge, they may be involved with the regulation of RNA synthesis and processing. Thus, the lamins could also be involved in mediating the effects of the expanded-repeat *FMR1* mRNA.

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NF-B Mediates TNF- Inhibitory Effect on Human COMT Gene Expression. *I.E. Tchivileva[1], R. Sitcheran[2], A.S. Baldwin[2], W. Maixner[1], L. Diatchenko[1].* 1) Center for Neurosensory Disorders, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC.

Catechol-O-methyltransferase (COMT) is a ubiquitous enzyme that catabolyzes catecholamines. Recent studies documented that reduction in COMT activity results in increased pain sensitivity in animal models, and human genetic variants coding for low COMT activity are associated with heightened pain sensitivity. Increased pain sensitivity is one of the characteristics of both the acute and chronic inflammation. We thus hypothesized that regulation of COMT expression under inflammatory conditions could contribute to development of inflammatory pain. In present study, we cloned a distal promoter of human COMT (P2-COMT) into a luciferase reporter vector and transiently transfected the construct into PC-12 cell line. COMT expression is controlled by two promoters, but only the P2-COMT is active in the human brain tissues. We demonstrated that proinflammatory cytokine tumor necrosis factor- (TNF-) down-regulated the activity of P2-COMT. RT-PCR and Western analyses of cell lysates confirmed decreased level of endogenous COMT after TNF- treatment at both mRNA and protein levels. Our findings showed that transcription factor NF-B, which plays a pivotal role in inflammation, mediated this TNF- effects. Specifically:(i) an optimal NF binding site was found by TFSearch analysis of P2-COMT sequence; (ii) cotransfection of p65 (RelA) inhibited expression of the P2-COMT reporter construct; (iii) TNF-/p65-induced repression of P2-COMT reporter construct could be reversed by the inhibitor of NF-B (IB) thus confirming the specific involvement of NF-B. The mechanism of NF-B suppression was assessed using electrophoretic mobility shift assay (EMSA) and P2-COMT mutants with impaired NF binding site. These results represent a first demonstration of COMT regulation by the NF-B pathway and suggest that inflammation can lead to enhanced pain sensitivity through decreased COMT expression.

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Long range PCR facilitates the identification of *PMS2* specific mutations. *M. Clendenning[1], H. Hampel[1], J. La Jeunesse[1], A. Lindblom[2], J. Lockman[1], M. Nilbert[3], K. Sotamaa[1], A. de la Chapelle[1].* 1) Human Cancer Genetics Program, Comprehensive Cancer Center, The Ohio State University, Columbus, OH; 2) Karolinska Institute, Department of Molecular Medicine, Stockholm, Sweden; 3) Department of Oncology, Clinical Sciences, Lund University, Lund, Sweden.

Mutations within the DNA mismatch repair gene, *PMS2* (postmeiotic segregation increased 2), have been associated with a predisposition to hereditary non-polyposis colorectal cancer (HNPCC; Lynch syndrome). Unlike the other members of the DNA mismatch repair family, accurate mutation detection within this gene has proved very difficult due to the presence of a large family of highly homologous *PMS2* pseudogenes. Here, we describe a novel method that utilizes long range PCR as a means to preferentially amplify *PMS2* and not the pseudogenes. A second, exon-specific, amplification from diluted long range products enables us to obtain good clean sequence that shows no evidence of pseudogene contamination. This method has been successfully utilized to screen a cohort of HNPCC-like patients (colorectal and endometrial cancers), whose tumors were negative for the PMS2 protein by immunohistochemistry, and had not shown any mutations within the *MLH1* gene. Sequencing of the *PMS2* gene from 30 colorectal and 9 endometrial patients identified 9 novel sequence changes as well as 16 sequence changes that had previously been identified. In total, putative pathologic mutations were detected in 9 of the 39 families. Among these were four novel mutations, c.705+1G>T, c.736_741del6ins11, c.1688G>T and c.2113G>A. The indel mutation within exon 7 was identified in two Swedish national patients and one American citizen who is of Swedish descent, and may potentially represent a Swedish founder mutation.

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ABCA1 mediated lipid transport from both the liver and the periphery are essential for maintenance of plasma HDL levels in vivo. *R. Singaraja[1], M. van Eck[2], C. Fievet[3], N. Bissada[1], M. Kang[1], L. Brunham[1], M. Hayden[1].* 1) CMMT,Univ British Columbia, Vancouver,Canada; 2) Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands; 3) INSERM, Institut Pasteur de Lille, Lille, France.

Mutations in ABCA1 underlie Tangier disease and familial hypoalphalipoproteinemia which are characterized by low plasma HDL-C. ABCA1 is expressed widely and its function in various tissues has not been ascertained. Until the discovery of ABCA1 as a gene essential for regulating plasma HDL-C homeostasis, cells in the periphery were considered critical for the regulation of HDL. This was challenged by recent data overexpressing ABCA1 specifically in the liver via adenoviral delivery, and also by selective deletion of ABCA1 in the liver (hepatic ABCA1-/-) which highlighted the primary role of the liver in maintenance of HDL levels. Mice with complete deletion of ABCA1 (TKO) and with selective deletion of ABCA1 in the liver (LSKO) allowed us to examine the discrete roles of ABCA1 in the liver and periphery and their relationship to HDL levels. Adenoviral delivery of ABCA1 resulted in selective expression of ABCA1 in the liver of both TKO and LSKO mice. Expression of ABCA1 in the liver of LSKO mice resulted in a significant increase in plasma HDL-C levels to that seen in wild-type mice (pre-infection:3.61.4, post-infection:38.31.3, wt:37.61.2, all mg/dL). However, expression of ABCA1 in the liver of TKO mice resulted in plasma HDL-C levels that were significantly lower than wild type levels (pre-infection:1.40.2, post-infection:19.24.3, wt:37.61.2, all mg/dL). The HDL particles from the ABCA1 expressing LSKO mice were larger than those from TKO mice (LSKO:11.90.4, TKO:10.30.5, nm, p=0.006). In addition, a significant increase in the concentration of large HDL particles was observed in the ABCA1 expressing LSKO mice (LSKO:52.47.1, TKO:19.28.9,mg/dL,p=0.003). These data indicate that ABCA1 expression in the liver is essential but insufficient to maintain normal plasma HDL, and indicate that the contribution of both liver and extrahepatic ABCA1 are essential for the regulation of plasma HDL levels.

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Molecular pathogenesis of mutations causing X-linked heterotaxy. *S.M. Ware, J. Purnell.* Cincinnati Children's Hospital Medical Center, Department of Pediatrics, Cincinnati, OH.

Members of the ZIC family of C2H2 zinc finger transcription factors are important mediators of patterning during development. Mutations result in a variety of brain, limb, cardiac, and visceral organ anomalies in humans and animal models. We have identified ZIC3 point mutations in patients with X-linked heterotaxy and also in patients with isolated congenital heart defects. Analyses of all known ZIC3 mutations demonstrate that loss of function results not only from impairment of transactivation ability but also from aberrant subcellular localization, suggesting that the pathogenesis of a subset of ZIC3 mutations results from failure of appropriate nuclear localization. The nucleocytoplasmic shuttling and transactivation properties of ZIC3 have been identified using a combination of in vivo nuclear export and nuclear localization assays, and GAL4-ZIC3 fusion domain mapping analyses. Zinc fingers 2-5, encompassing amino acids 290-420, are responsible for nuclear localization, nuclear export, DNA binding and physical interaction with GLI superfamily members. A cryptic CRM1-dependent nuclear export signal is present between amino acids 290-350 of ZIC3. The missense mutation T323M results in the unmasking of this nuclear export signal such that the mutant protein is cytoplasmic. Correction of aberrant ZIC3 cytoplasmic localization does not restore transactivation potential, highlighting the complex functions of this protein domain. Mapping of the nuclear localization domain of ZIC3 indicates it spans a relatively large region from amino acids 290-420, and overlaps with the GLI binding region. ZIC3 and mutant ZIC3 co-localize with GLI3 but not GLI2 in subcellular localization assays, suggesting that ZIC3-GLI3 may shuttle to the nucleus as a complex. These results provide the first evidence that ZIC3 is a nucleocytoplasmic shuttling protein and indicate that aberrant shuttling underlies a subset of X-linked heterotaxy cases. Given that this region is highly conserved among ZIC family members, defects of nucleocytoplasmic shuttling may contribute to a broader spectrum of congenital anomalies.

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Exaggerated Blood Pressure Response to Exercise in Epinephrine-Deficient Mice. *X. Bao[1], C.M. Lu[2], Y. Gu[1], F. Liu[1], J. Chen[1], P.C. Simpson[3], M.G. Ziegler[1].* 1) Dept. of Medicine, University of California at San Diego, CA; 2) Dept. of Labratory Medicine, VA Medical Center at San Francisco, CA; 3) Dept. of Medicine, VA medical Center at San Francisco, CA.

Epinephrine (E) release increases during exercise along with elevated heart rate (HR) and blood pressure (BP). To better understand the role of E in the cardiovascular adaptation to exercise, we generated a mouse model whereby the E biosynthesizing enzyme phenylethanolamine n-methyltransferase (PNMT) has been knocked out (KO). The E levels in various tissues including plasma and urine were undetectable in PNMT KO mice. Mean arterial blood pressure (MAP) and heart rate (HR) in KO (n=12) and wild type (WT, n=11) mice were telemetrically monitored at rest and during graded treadmill exercise, initiated at 0.06 m/s, followed by 0.03 m/s increase every 2 min for 12 min. Although 24-hour MAP and HR were maintained at normal levels in resting PNMT KO mice, their MAP responses to initial exercise at 0.06 m/s were significantly increased by 37.9% to 137.93.8 mmHg compared to WT controls (increase by 12.3% to109.77.2 mmHg, P=0.002). This excessive increase in MAP remained through exercise session at nearly every speed. However, there was no difference in increase in heart rate responses to exercise between KO and WT mice. Furthermore, the exercise capacity was significantly reduced in KO mice compared to WT (4.11.63 versus 0.270.1 breaks/min at 0.21 m/s,P<0.05; maximum speed 0.170.01 versus 0.210.01 m/s, P<0.05). Our data suggest that E is required to offset exaggerated BP response to exercise but not required for the increased HR during exercise.

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Reproducing the haplotype; the role of the 5-lipoxygenase activating protein in ischaemic heart disease in Ireland. *P.G. Horan[1], A.R. Allen[2], A.E. Hughes[3], C.C. Patterson[4], M.S. Spence[1], P.G. McGlinchey[1], C. Belton[2], P.P. McKeown[1 2].* 1) Regional Medical Cardiology Centre, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA, Northern Ireland, UK.United Kingdom; 2) Department of Medicine, Queens University Belfast, Institute of Clinical Science, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK; 3) Department of Medical Genetics, Queens University Belfast, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK; 4) Department of Epidemiology and Public Health, Queens University Belfast, Mulhouse Building, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK.

Introduction. Low-density lipoprotein (LDL) oxidation by leukotrienes and the 5-lipoxygenase activating protein (FLAP) plays a central role in atherosclerosis. A four single nucleotide polymorphism (SNP) haplotype (HapB) in the gene encoding FLAP has been shown to be associated with myocardial infarction (Helgadottir et al. Nature Genetics, 2004). Within HapB, SNP SG13S114 has been shown to be associated with stroke (Lohmussaar et al. Stroke, 2005). We investigated HapB in an Irish study group with premature ischaemic heart disease (IHD) (males 55 years, females 60 years). Methods. 1494 individuals (580 families) were included. 10 individuals (3 families) were excluded due to insufficient DNA, leaving 799 discordant sib-pairs and 62 parent-child trios. Linkage disequilibrium between HapB and IHD was tested using the combined transmission disequilibrium test and pedigree disequilibrium test. Results. SNP SG13S377 was excluded as it showed no heterogeneity in our study group. The numbers of informative families for SG13S114, SG13S41 and SG13S35 were 271, 79 and 127, respectively. A significant association between the informative SNPs of HapB and IHD was detected (p=0.032) due to the preferential transmission of the T allele of SG13S114 (p=0.015). Conclusions. Using family-based association tests we have shown association between the FLAP gene (particularly SG13S114) and IHD. Replication of previous work suggests an important role for FLAP in the pathogenesis of atherosclerosis.

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Alternative splicing appears to be not responsible for the appearance of protein isoforms of eukaryotic elongation factor-2 (EEF2) in human heart. *S. Schulz[1], C. Glaeser[2], J. Holtz[3], K. Werdan[1], U. Mueller-Werdan[1].* 1) Departm Internal Med, Univ Halle, Halle, Germany; 2) Inst Human Genetics, Univ Halle, Halle, Germany; 3) Inst Pathophysiology, Univ Halle, Halle, Germany.

The eukaryotic protein biosynthesis is catalyzed by a variety of interacting enzymes. In the healthy heart the last step of the elongation cycle catalyzed by the eukaryotic elongation factor 2 (EEF2), a 95 kDa monomeric G-protein, is considered to play a major regulatory role. In previous studies it could be shown that this enzyme occurs in various protein variants showing different biological activity under physiological conditions. Up to now, posttranslational modifications including phosphorylation and ADP-ribosylation are considered to be the main cause for the different isoforms of EEF2 and its regulation under varying metabolic circumstances. The aim of the present study was to investigate, whether possible modifications in the processing of the mRNA may also account for the existing isoforms. Therefore, we isolated RNA from heart samples obtained from 8 human donors using TriStar-reagentTM. EEF2 mRNA was reversibly transcribed using specifically designed primers. For investigating the size of EEF2 transcripts four overlapping PCRs were performed using primer combinations suitable to span the complete mRNA. However, for none of the 8 tissue samples of human heart any variant mRNA fragment was detected. This result supports the thesis, that not differences in the splicing procedure but posttranslational modifications are responsible for the appearance of EEF2 isoforms at least in the human heart.

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The establishment of a predictive mutational model of the Forkhead domain through the analyses of FOXC2 missense mutations identified in patients with hereditary lymphedema with distichiasis. *M.A. Walter[1,2], Y. Tamimi[2], M. Carle[2], O.J. Lehmann[1,2], F.B. Berry[2].* 1) Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Depart Ophthalmology, Univ Alberta, Edmonton, AB, Canada.

The FOX family of transcription factor genes is an evolutionary conserved, yet functionally diverse class of transcription factors that are important for regulation of energy homeostasis, development and oncogenesis. The proteins encoded by FOX genes are characterized by a conserved DNA-binding domain known as the Forkhead domain. To date disease-causing mutations have been identified in eight human FOX genes. Many of these mutations result in single amino acid substitutions in the Forkhead domain. We analyzed the molecular consequences of two disease-causing missense mutations (R121H and S125L) occurring in the Forkhead domain of the FOXC2 gene that were identified in patients with hereditary lymphedema with distichiasis to test the predictive capacity of a forkhead domain structure/function model. Based on the FOXC2 solution structure, both FOXC2 missense mutations are located on the DNA-recognition helix of the forkhead domain. A mutation model based on the parologous FOXC1 protein predicts that these FOXC2 missense mutations will impair the DNA-binding and transcriptional activation ability of the FOXC2 protein. When these mutations were analyzed biochemically, we found that both mutation did indeed reduce the DNA-binding and transcriptional capacity. In addition the R121H mutation affected nuclear localization of FOXC2. Together these data indicate that these FOXC2 missense mutations are functional nulls, that FOXC2 haploinsuffeciency underlies hereditary lymphedema with distichiasis, and validates the predictive ability of the FOXC1-based Forkhead domain mutational model.

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Epigenetic silencing of retrotransposed L1s in cultured mammalian cells. *J.L. Garcia-Perez[1], K.S. O'Shea[2], J.V. Moran[1].* 1) Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 2) Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

L1s are abundant retrotransposons that comprise ~17% of human DNA. The average human genome is estimated to contain ~80-100 retrotransposition-competent L1s (RC-L1s) and their mobility can result in disease. The proteins encoded by RC-L1s also are responsible for the mobilization of Alu elements and the formation of processed pseudogenes, which comprise at least 10% of human DNA. Thus, L1 retrotransposition has had a tremendous impact on human genome evolution. Despite these findings, little is known about the host mechanisms that act to promote or restrict L1 retrotransposition. Using a series of engineered L1s, we have demonstrated that human and mouse embryonic stem cells (hES and mES) as well as human ovarian teratocarcinoma cells can accommodate L1 retrotransposition. L1 retrotransposition in these cell types appears to be relatively inefficient when compared with other human cultured cells (i.e., HeLa cells). In addition, our data indicate that the new L1 retrotransposition events in both ovarian teratocarcinoma and ES cells undergo epigenetic silencing either during or soon after integration, and that this silencing can be reversed by treating the cells with histone deacetylase inhibitors (HDAC). Further experiments in ovarian teratocarcinoma cells revealed the following: 1) silencing can be reversed with several HDAC inhibitors; 2) silencing does not depend on the promoter or poly (A) signal used to express the retrotransposed reporter gene; 3) silencing also occurs for retrotransposition events derived from a synthetic mouse L1. Thus, we hypothesize that epigenetic silencing might represent a host mechanism to restrict the number of retrotransposition-competent LINE-1 sequences in mammalian cells.

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Functional Characterization of the *Hind*III Polymorphism in the Lipoprotein Lipase Gene. *Q. Chen, W.J. Xu, H. Razzaghi, M.I. Kamboh.* Human Gen, Grad Sch Pub Hlth, Univ Pittsburgh, Pittsburgh, PA.

Lipoprotein Lipase (LPL) plays a pivotal role in lipid metabolism by hydrolyzing triglyceride (TG) rich liporprotein particles. Abnormalities in normal LPL function are associated with the risk of coronary artery disease (CAD). A number of genetic variants have been identified in the LPL gene that affect different functions of the LPL protein. A common *Hind*III polymorphism in intron 8 (T/G) of the LPL gene has been found to be associated with altered plasma TG and HDL-cholesterol, and CAD risk in several studies, but its functional significance is unknown. It has been shown that certain intronic sequence contain regulatory elements that are important for transcription and translational regulation of a gene. In this study we tested the hypothesis that the T/G polymorphism at the *Hind*III site affects the binding site of a transcription factor that regulates the transcription of the LPL gene. Electrophoretic mobility shift assays indicated that the *Hind*III site binds to a transcript factor and that the wild type allele has higher binding affinity than the mutant allele. For transcription assay, entire intron 8 DNA (1,392 kb) fragment containing the wild type (T) or mutant type (G) *Hind*III site was subcloned upstream of the luciferase gene in pGL3-basic vector and cotransfected COS-1 cells along with *Renila* Luc control vector (pRL-TK). Full-length LPL promoter (1,537 bp) was also inserted upstream of the intron 8 fragment in the expression vector to enhance the gene transcription. Transfected cells were harvested after 48 hours and lysed in the lysis buffer followed by measurement of luciferase activities using the dual luciferase assay system. Data were obtained from three independent experiments, each performed in triplicates. Our results showed that the mutant allele at the *Hind*III site significantly decreased the luciferase expression level compared to the wild type allele (3.38 ¡À 1.21 vs. 4.30 ¡À 1.31; p = 0.037). This study demonstrates that the polymorphic *Hind*III site in the LPL gene is functional because it affects the binding of a transcription factor and it also has impact on LPL expression.

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Mutations in the MTO2 related to tRNA modification impair mitochondrial gene expression and protein synthesis in the presence of a paromomycin resistant mutation in mitochondrial 15S rRNA. *M.X. Guan, Q. Yan, X. Li.* Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH.

Nuclear gene(s) has been show to modulate the phenotypic expression of mitochondrial DNA mutations. We report here the identification and characterization of the yeast nuclear gene MTO2 encoding an evolutionarily conserved protein involved in mitochondrial tRNA modification. Interestingly, mto2 null mutants expressed the respiratory deficient phenotype when coexisting with the C1409G mutation of mitochondrial 15S rRNA, at the very conservative site for human deafness-associated 12S rRNA A1491G and C1409T mutations. Furthermore, the overall rate of mitochondrial translation was markedly reduced in a yeast mto2 strain under the wild type mitochondrial background, while the mitochondrial protein synthesis was almost abolished in a yeast mto2 strain carrying the C1409G allele. The other interesting feature of mto2 mutants is the defective expression of mitochondrial genes, especially in CYTB and COX1, only under the coexistence of the C1409G allele. These data strongly indicate that a product of MTO2 functionally interacts with the decoding region of 15S rRNA, particularly at site of C1409G or A1491G mutation. In addition, we showed that yeast and human Mto2p localize in mitochondria. The isolated human MTO2 cDNA can partially restore the respiratory deficient phenotype of yeast mto2 cells carrying the C1409G mutation. These functional conservations imply that human MTO2 may act as a modifier gene, modulating the phenotypic expression of the deafness-associated A1491G or C1409T mutation in mitochondrial 12S rRNA.

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The Effects of d337T Thyroid Hormone Receptor (TRb) and Peroxisome Proliferator Actived Receptor (PPARa) on Early Responding Cardiac Gene Expression. *N.E. Buroker[1], M.E Young[2], C. Wei[3], K. Serikawa[4], M. Ge[1], X-H. Ning[1], M.A. Portman[1].* 1) Department of Cardiology, Seattle Childrens Hospital & Regional Medical Center, 4800 Sand Point Way N.E., Seattle, WA 98105; 2) Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030; 3) Department of Biostatistics and Applied Mathematics, University of Texas M.D.Anderson Cancer Center, Houston, TX 77030; 4) Center for Expression Arrays, Department of Microbiology, University of Washington, School of Medicine, Seattle, WA 98195.

In this study we used the d337T TRb transgenic mouse that has been created to reproduce the human genetic disease known as resistance to thyroid hormone(RTH)as a model to determine if the d337T TRb mutation would have an effect on PPARa activation. A single amino acid deletion(d337T) abrogates thyroid hormone(T3)binding and transforms the thyroid hormone receptor(TRb)into a constitutive repressor. The principle goal was to determine if T3 regulates myocardial energy metabolism through its nuclear receptors. We introduced a known PPARa activator(WY14,643)into control and d337T TRb mice then examined cardiac gene expression using Affymetrix 430_2 GeneChips and RT-PCR. We compared the gene expression of PPARa,RXRb & TRa,b and 3 PPARa target genes among 4 studies groups [cont, cont+WY14,643,d337TTRb,& d337TTRb+WY14,643]. Microarray analysis revealed that these genes responded to the WY14,643 treatments of control and d337T TRb mice. Analysis of the array and RT-PCR data indicates that mRNA expression levels of PPARa and mRXRb decrease after a 6 hour drug treatment in both control and d337T TRb mice ($P<0.01$) as did the array mRNA expression levels for TRa & b($P<0.025$). Three target genes (AMPD3, PDK4 and UCP3) of PPARa were up regulated in control and down regulated in the d337T TRb mouse,indicating a direct action on these metabolic genes when the TRb becomes a repressor. In conclusion, PPARa activation by WY14, 643 has a positive effect on control mice and a negative effect on the TRb transgenic mice which supports our hypothesis that T3 regulates myocardial energy metabolism through its nuclear receptors.

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Visualization of functional human gene domains in nuclear chromatin. *P. Sabo[1], S. Kuehn[2], W. Noble[3], M. Dorschner[1], J. Stamatoyannopoulos[1].* 1) Dept of Molecular Biol, Regulome, Seattle, WA; 2) Div. of Medical Genetics, University of Washington, Seattle, WA; 3) Dept. of Genome Sciences, University of Washington, Seattle, WA.

The functional organization of the human genome is reflected in the topology of nuclear chromatin. We recently described a high-throughput chromatin accessibility assay - Quantitative Chromatin Profiling (Nature Meth. 1:219-25, 2004) - that permits large-scale quantitative determination of chromatin structure in vivo. We have now applied this approach to define at high resolution the chromatin architecture of several human multi-gene loci including the beta-globin locus (Chr11); IL3/4/5/13 locus (Chr5); CFTR locus (Chr7); ApoA1/A4/A5/C3 locus (Chr11); alpha-globin locus (Chr16); IGF2/H19 locus (Chr11); HoxA2 locus (Chr11); and the Down Syndrome critical region (Chr21). These studies reveal a vibrant patchwork of interleaved 'open' and 'closed' chromatin domains that organize functionally related genes. Surprisingly, these domains only loosely correspond with gene expression status and appear to dominate more localized patterns of histone acetylation and methylation. In many cases functional domains in chromatin parallel long-range patterns of evolutionary conservation, and frequently display a striking inverse correlation between chromatin accessibility and the density of conserved non-coding sequences. This suggests that many of the functional elements regulating chromatin accessibility - and hence the access of critical transcriptional regulatory factors to the genome - are of recent evolutionary vintage.

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Gene profile analysis of typical and atypical disease states in Friedreich's Ataxia. *D. McDaniel[1], S.H. Subramony[2], L.S. McDaniel[3].* 1) Surgery, University of MS Medical Center, Jackson, MS; 2) Neurology; 3) Microbiology.

Since the discovery of the Friedreichs Ataxia gene known as X25, located on chromosome 9 numerous progress have been made towards the identification of the elements associated with the pathogenesis of the disease. It is well established that a majority (97%) of patients with Friedreichs ataxia (FA) carry a mutation associated with expansion of GAA.TCC trinucleotide repeats in the first intron of the X25 gene. The mutation is associated with defects in the FA gene coding for the iron storage protein frataxin. Multiple models have been proposed regarding to the dynamic formation of DNA triplexes (referred to sticky DNA) and its association with the reduction in mRNA transcription. In this study we investigate the scope of the mRNA and signal pathways affected by FA gene in typical and atypical disease states using focus microarrays. Patients were identified by their clinical presentation and size of GAA expansion. The PBMCs were used for evaluation of gene expression of neurotrophin and receptors signaling pathways in GEAarrays. We performed gene expression profiling of 4 samples, 2 typical FA with expansion repeat size of equal or greater than 800 and 2 atypical patients with expansion size approximately 130 repeats. Signal values were normalized by dividing by the relative expression level of the housekeeping gene in the same sample. Using the normalization criteria at least 9% of the transcripts showed significant reduction in typical FA samples as compared with atypicals. Activating transcription factor 2 (ATF-2), nerve growth factor receptor (TNFR), neurotrophic tyrosine kinase receptor, type 1 and 2, signal transducer and activator of transcription 3 (STAT3), STAT5 and TAC1 were expressed higher in atypical FA as compared with typical FA patients. In summary, this study might generate new diagnostic parameters to test transcriptional differences between disease states and level of gene mutation.

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ATM splicing mutation 5932 G > T decreases SRp40 binding to an ESE motif. *S.E. Arroyo, H. Feng, R. Gatti.* The David Geffen School of Medicine at UCLA, Department of Pathology and Laboratory Medicine, Los Angeles, CA, 90095-1732, USA.

Ataxia Telangiectasia (AT) is a progressive, neurodegenerative disease caused by mutations in the ATM gene. Over 400 unique mutations have been identified, none with a frequency greater than 3%. Of these unique mutations, approximately 85% result in truncated protein created by premature termination codons. An interesting subset of these mutations are splicing defects that can appear as nonsense, missense, or even silent mutations. One such splicing mutation is the nucleotide substitution 5932 G > T, 14 nucleotides from the beginning of exon 42. Although the mutation encodes for a primary termination codon - GAA (Glu) TAA (ter) - the mRNA transcript reveals that the consequence is in fact the skipping of exon 42. While it is unlikely that the mutation disrupts a splice junction motif because of its nested position within the exon, analysis of exonic splicing enhancer (ESE) motifs reveals that a binding site for SRp40 is also impacted by this mutation. We designed a protein pull-down assay to test whether this mutation affects the binding of SRp40. Small biotinylated RNA oligonucleotides were made for both wild type and mutant sequences with the substitution near the midpoint. The oligonucleotides were then fixed onto streptavidin-coated magnetic beads and incubated in nuclear lysates. Eluates from these beads were run on SDS-PAGE gels, probed with antibodies to SR proteins, and compared for differential band intensities. Our results show that the SRp40 band, seen clearly in eluates from wild type oligonucleotides, was diminished or absent in eluates from mutant oligonucleotides. We conclude that the primary disease-causing mechanism in this ATM mutation is the disruption of the splicing function of an ESE motif.

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Functional study of a novel 5 bp deletion mutation located in a putative insulin response element in exon 10 of human lipoprotein lipase gene. *L.-X. Yang[1], H. Razzaghi[2], M.I. Kamboh[1].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Preventative Medicine, Univ Colorado, Denver, CO.

Lipoprotein lipase (LPL) plays a pivotal role in lipid metabolism and abnormalities in LPL function are associated with coronary heart disease. LPL gene is sensitive to hormonal regulation of insulin, but the insulin response element (IRE) has not been identified. Previously we identified a 19 bp putative IRE in non-coding exon 10 of LPL and a 5 bp naturally occurring deletion within the putative IRE. The purpose of this study is to determine if the proposed IRE is indeed a functional motif, to identify transcriptional factor that binds to it and to test if the 5 bp deletion affects LPL gene expression and mRNA stability. Competitive Gel shift assay demonstrated that a nuclear protein binds to the putative IRE in non-coding exon 10 in a sequence-specific manner. To identify the nuclear factor binding to the putative IRE, we performed antibody supershift assay with the transcription factor antibodies. When using human aorta smooth muscle cell nuclear extract instead of Hela cell nuclear extract, a supershifted DNA-protein-antibody complex was found in the presence of Elk-1 antibody. These results indicate that transcription factor Elk-1 specifically binds to the putative IRE or evolutionarily conserved sequence in LPL gene. To clarify the functional significance of the 5 bp deletion, the mutant and wild type cDNA expression plasmids were transfected into COS-1/human muscle cells, treated with or without insulin and followed by TaqMan real-time RT-PCR to quantitatively measure the changes of LPL gene expression. We did not see significantly reduced mRNA in the deletion mutant expressions. Further in vitro experiments testing the influence of the deletion on mRNA stability are in progress. We also constructed the luciferase expression vectors containing the human LPL promoter and full-length cDNA with or without the 5 bp or 19 bp deletion in the putative IRE to examine LPL promoter activities. We are performing Western blot analysis to determine if the mutation affects LPL gene expression in response to insulin at the translational or post translational (phosphorylation) level.

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Interaction between IL1B gene promoter polymorphisms in determining susceptibility to Helicobacter pylori associated duodenal ulcer. *M. Chakravorty, A. Choudhury, S. Roychoudhury.* Human Genetics and Genomics, Indian Institute of Chemical Biology, Kolkata, West Bengal, India.

The variability in the clinical outcome of the host upon *Helicobacter pylori* infection is attributed to both bacterial as well as host genetic factors. It has been proposed that single nucleotide polymorphisms present in the cytokine genes are responsible for the alteration in expression of these genes.The pro inflammatory cytokine IL1B plays a key role because of its strong inhibitory role towards gastric acid secretion in the gastric mucosa, upon infection with *H.pylori*. Thus to understand the contribution of host immunogenetics towards *H.pylori* mediated duodenal ulcer,310 individuals from Eastern India were subjected to a case control study to determine the *IL1B*and *IL1RN* risk genotypes to *H.pylori* mediated duodenal ulcer.Analysis of genotype frequency revealed significantly higher frequency of *IL1B*-511T/T, OR=4.22 (95% CI 1.8-9.5) and -31 C/C, OR=2.16 (95% CI 1.12-4.16) genotypes in *H.pylori* infected individuals with duodenal ulcer compared to infected individuals with normal mucosa. Quantitative analysis of the mucosal IL1B mRNA revealed that among *H.pylori* infected individuals, carriers of the -31C/C genotype, had significantly lower IL1B transcript levels than carriers of C/T and T/T genotypes. To dissect the role of these allelic polymorphisms in an in vitro model we carried out IL1B promoter assay, which showed that promoter with -31C, had a 10-fold lower activity when compared to the -31T. Moreover, IL1B promoter bearing the different combinations of both the polymorphic loci showed interaction between the -511 and -31 loci. The effect of allelic variation of IL1B upon acid secreting gene gastrin was studied in detail, which suggested that the allele conferring lower transcriptional ability also led to lesser inhibition of gastrin. Our results show that *H.pylori* infected individuals with -31C/C genotype secrete less amount of IL1B, which in turn leads to lesser inhibition of gastrin, and hence are susceptible to duodenal ulcer. We also propose that allelic interaction between the -511 and -31 polymorphic sites determines the overall strength of the IL1B promoter.

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A splicing variant of the human lysyl oxidase-like gene 3 (*LOXL3*) functions as an amine oxidase with distinct tissue-specificity from *LOXL3*. *Y. Kim[1, 2], J.E. Lee[2], S.Y. LEE[2], J.Y. Park[2].* 1) Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Diseases, University of Ulsan College of Medicine, Seoul, Korea.

The lysyl oxidase-like gene 3 (*LOXL3*) encodes a member of the emerging family of lysyl oxidase (LOX) that functions as a copper-dependent amine oxidase catalyzing lysine-derived cross-links in extracellular matrix. The LOXL3 protein contains four scavenger receptor cysteine-rich (SRCR) domains in the N-terminus in addition to the characteristic domains of the LOX family such as the copper-binding domain, the cytokine receptor-like domain and the residues of the lysyl-tyrosyl quinine cofactor in the C-terminus. Using a BLASTN search with the *LOXL3* cDNA sequence, we identified an EST clone that contained the cDNA sequence of *LOXL3* from exons 4 to 14 but lacked the sequence corresponding to exon 5. This *LOXL3* splicing variant (*LOXL3*-ex5) showed a distinct exon-intron structure from *LOXL3*, containing a 90 bp sequence corresponding to intron 3 of *LOXL3* in the 5 UTR and a 561 bp sequence corresponding to the 3 proximal genomic region of exon 14 in the 3 UTR. *LOXL3*-ex5 was predicted to encode a protein of 393 amino acids, containing the C-terminal domains required for the amine oxidase activity but lacking the N-terminal SRCR domains 1 and 2 of *LOXL3*. To assess its amine oxidase activity, we expressed the LOXL3-ex5 protein as a recombinant form attached with hexa-histidine residues at the C-terminus, using an *Escherichia coli* expression system. The purified LOXL3-ex5 protein showed -aminopropionitrile-inhibitable activity of amine oxidase toward collagen type I. As analyzed by RT-PCR assays with various human tissues, *LOXL3*-ex5 was predominantly expressed in the pancreas, spleen, thymus, and liver, while *LOXL3* was more specifically expressed in the heart, placenta, liver and lung. Luciferase reporter assays revealed an orientation-dependent promoter element in the 5 proximity of intron 3. These findings indicate that the differently spliced mRNAs of *LOXL3*, showing distinct tissue specificity, arise as a consequence of alternative promoter activation.

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Gene expression profiling of murine spermatogenesis leads to the identification of mArd2, a novel mouse Ard1 homolog that is preferentially expressed starting from meiosis. *A.L.Y. Pang[1], W. Johnson[1], D.H. Bear[1], O.M. Rennert[1], W.Y. Chan[1,2].* 1) Lab of Clinical Genomics, NICHD/NIH, Bethesda, MD; 2) Cell Biology and Pediatrics, Georgetown University, Washington, DC.

Spermatogenesis is a tightly regulated developmental process of the male germ cells. The concomitant change in morphology during germ cell differentiation highly suggests an underlying global change in gene expression pattern. We profiled the transcriptomes of different stages of male mouse germ cells to elucidate their functional differences. From that, an EST showing preferential expression in meiotic germ cells was chosen for characterization. Subsequent RACE experiment led to the identification of a 3.4-kb transcript encoding a putative homolog of N-acetyltransferase subunit mArd1, which is named mArd2. In silico analysis revealed the existence of three highly conserved Ard1 homologs in the mouse genome. Interestingly, differential expression patterns were observed for these Ard1 homologs in various tissues and different stages of germ cells, with mArd2 showing a testis-specific expression pattern. The use of alternative transcriptional start sites and polyadenylation signals in mArd2 transcripts was observed in different stages of spermatogenesis. A mArd2-specific antibody preparation was generated. Preliminary Western blotting and immunohistochemistry analyses verified the germ cell-specific expression pattern of mArd2. However, the highest level of mArd2 was detected only in post-meiotic germ cells, indicating the mArd2 transcript was translationally active only after meiosis. We also predicted the presence of specific regulatory motifs, which were recently shown to be miRNA binding sites, on the 3 untranslated region of mArd2. Our results suggest that mArd2 is responsible for a germ cell-specific function and its expression may be regulated at both transcriptional and post-transcriptional levels. The yeast ARD1 has been implicated in functions such as cell cycle regulation, DNA repair/recombination and histone acetylation. Further characterization experiments are underway to elucidate the functional role of mArd2 in germ cell development.

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Common Human Catechol-O-methyltransferase Haplotypes Modulate RNA Stability, Protein Expression, and Enzymatic Activity. *A.G. Nackley[1], S.A. Shabalina[2], W. Maixner[1], L.B. Diatchenko[1].* 1) Ctr for Neurosensory Disorders, University of North Carolina, Chapel Hill, NC; 2) National Center for Bioinformatics, NIH, Bethesda, MD.

Catechol-O-methyltransferase (COMT) has recently been implicated in the modulation of persistent pain. The COMT gene encodes two proteins: the soluble form (S-COMT), expressed mainly in peripheral tissues, and the membrane-bound form (MB-COMT), the exclusive form expressed in the central nervous system. We demonstrated that three common haplotypes of the human COMT gene are associated with pain sensitivity and the risk of developing temporomandibular disorder (TMD)(Diatchenko et al., HMG 2005). Based on subjects pain ratings, COMT haplotypes were designated as low (LPS), average (APS), or high pain sensitive (HPS). Individuals with HPS and APS haplotypes were ~2.5 times more likely to develop TMD. Furthermore, reductions in COMT activity result in elevated pain sensitivity in rats (Nackley et al., SfN 2004). Thus, a clear link exists between COMT and pain. The present study was conducted to elucidate the mechanism whereby COMT haplotypes modulate pain sensitivity. The relationship between S- and MB-COMT haplotype, RNA stability, protein expression, and COMT enzymatic activity was evaluated in the lysate of HEK 293 cells transiently transfected with identical full-length cDNA clones corresponding to the three haplotypes. COMT RNA stability, protein expression, and enzymatic activity were measured using Real-Time PCR, western blotting, and normetanephrine ELISA, respectively. Total S- and MB-COMT RNA were significantly greater in cells expressing the LPS haplotype relative to the APS and HPS haplotypes. Similarly, cells expressing the LPS haplotype exhibited the highest amount of S- and MB-COMT enzymatic activity and protein, while those expressing the APS or HPS haplotype displayed significantly lower levels. Taken together, these results suggest that HPS and APS COMT haplotypes produce heightened pain sensitivity by coding for reduced total RNA which ultimately results in decreased levels of the corresponding protein and enzyme activity. Support: DE007333 to AN, DE016558 to LD, NS045685 to WM.

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Ala and Glu are the amino acids at 360 and 654 sites of the PDEB in Mexican population. Until now considered regions in conflict. *C. Córdova, R.A. Lara, I.C. Núñez, C. Medina.* Genética, CIBO (IMSS), Guadalajara, Guadalajara, Mexico.

Introduction. The beta subunit of the cGMP phosphodiesterase (PDE6B) participates in the processes of transmission and amplification of the visual signal in rod cells. PDE6B has two cGMP-binding sites (GAF-I and GAF-II) in the N-terminal half and a catalytic domain (PDEase) in the C-terminal portion. The PDEB gene (4p16.3) presents seven regions in conflict. Regions in conflict are those with more than one version of the nucleotide sequence encoded by a specific DNA region. The conflict at site 360 in the protein PDE6B is caused by putative assignment of the amino acids alanine (Ala) and arginine (Arg) coded by GCT and CGT triplets, respectively, it located in the exon 8 from GAF-II domain. The conflict at site 654 is caused by assignment of the amino acids glutamic acid (Glu) and aspartic acid (Asp) coded by GAG and GAT triplets, respectively, it located in the exon 16 from PDEase domain. Material and Method. We analyzed PCR products of the 8 and 16 exons from 50 healthy individuals, using the BsmI and BssSI restriction enzymes that recognize to GAATGCNNN and GTGCTC complementary of GAG. Results. The GCT and GAG triplets were present in the 100 chromosomes, showing that Ala and Glu are universally present at 360 and 654 sites of the protein PDE6B. Discussion and Conclusion. Ala and Arg are amino acids with dissimilar polarity that would involve different function in the protein. Ala 360 is an amino acid higher conserved in the PDEB of other species. The 654 site shows several amino acids with different biochemical properties, It is probably that 654 site is not important for PDE6B protein function. We showed that Glu is the 654 amino acid present in the PDEB of Mexican population studied. Therefore, the 360 and 654 sites of PDEB must be not considered regions in conflict on the human PDEB appointment.

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Large-scale identification of transcription start sites and splicing of human genes using full-length cDNAs constructed by oligo-capping method. *T. Isogai[1], A. Wakamatsu[1], K. Kimura[2], J. Yamamoto[1], N. Nishikawa[1,2], Y. Suzuki[3], S. Sugano[3].* 1) Reverse Proteomics Res Inst, Kisarazu, Chiba, Japan; 2) Central Research Laboratory, Hitachi Ltd., Tokyo, Japan; 3) Department of Medical Genome Sciences, Graduate school of Frontier Sciences, University of Tokyo, Tokyo, Japan.

Human gene number was estimated to be 20-25 K. However number of human mRNA variety was predicted to be about 100 K. The varieties are thought to be caused by variations of transcription start site (TSS) and splicing. In our NEDO human cDNA project, about 30 K of human full-length sequenced cDNAs (FLJ cDNAs) were deposited to DDBJ/GenBank/EMBL, and we obtained about 1.4 million of 5'-onepass sequences (5'-onepass) of full-length cDNAs from about 100 kinds of cDNA libraries consist of human tissues and cells constructed by oligo-capping method. About 500 bp of all 5'f-end of the cDNAs were obtained. The majority of the insert cDNA sizes were over 2 kb and the full-length rate of 5'-end was 90%. And about 19 K of full-length sequenced FLJ cDNAs was newly obtained, major part of which were selected focusing on TSS and splicing variations. In this study we estimated TSS variations of human genome using our 5'-onepass cDNA sequences. We obtained 54,723 loci which 5'-onepass sequences of our cDNAs completely match to human genome sequences (Build 35). Firstly, using this we clustered cDNAs based on first exons to predict TSSs roughly. 45,907 of the reliable first-exon variations were obtained in 19,194 loci of human genome. Secondly, we clustered cDNAs under the conditions which permit even the difference in one base to predict TSS in detail. 70,529 of the reliable TSSs were obtained in 19,194 loci. From these results, the number of TSS was predicted to be approximately 50,000-70,000. This result was supported efficiently by 1.4 million of our 5'-onepass sequences. Then we also predicted TSSs of 2,094 clusters of multiple variable first exons. Furthermore, we found some TSS and splicing variations which had specific expression or motif. This work was supported by a grant from New Energy and Industrial Technology Developmental Organization (NEDO) project of the Ministry of Economy, Trade and Industry of Japan.

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Gene expression analysis in amplification/deletion region detected by array CGH method. *Y. Satoh[1], T. Tsuyama[2], M. Kodaira[1], N. Takahashi[1].* 1) Dept. Genetics, RERF, Hiroshima, Japan; 2) Appl. Med. Eng. Sci., Yamaguchi Univ., Ube, Japan.

We have studied the effects of atomic bomb radiation on human germ cells. Using the array CGH(comparative genomic hybridization) method to detect mutations occurring in the children of A-bomb survivors due to radiation, a number of amplification/deletion regions transmitted from parents to offspring were detected (Takahashi et al., present at this meeting). Realtime PCR assay was used to analyze whether expression levels of genes residing in such a genomic amplification/deletion region were correlated with genome amplification/deletion. A variant mapped to 18q23 was a 150 kb amplification with a structure of two 75 kb tandem duplicate sequences. AK056304 is a gene whose full length is included in the amplification region. PARD6G and KIAA0863 are genes whose partial length is included in the amplified region. Gene expression of AK056304 was increased in mother and child with this variant form compare with father with a normal form of the gene. Whereas KIAA0863 showed no difference in expression among father, mother and child regardless of the presence or absence of this variant form, PARD6G showed a significant decrease of mRNA expression in mother and child with amplified region. In this region, gene expression may be amplified by genome amplification. As we perform analysis of gene expression in other amplification/deletion regions, we are continuing the analysis of PARD6G, a gene whose expression level decreased despite the genome amplification.

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Identification and characterization of a novel transcript embedded in the *HNF4A* gene. *A.B. Lehtinen, F. Segade, J.C. Mychaleckyj, D.W. Bowden.* Wake Forest Univ Sch Med, Winston-Salem, NC.

The transcription factor *HNF4A* plays a key role in glucose, cholesterol, and fatty acid metabolism, and has been implicated in type 2 diabetes (T2DM) susceptibility. Control of *HNF4A* expression is complex, with two promoters separated by 45.5 kb driving transcription in a tissue-specific manner. While the promoter elements have been identified and characterized, little is known about the impact the intervening genomic sequence has on gene expression. Bioinformatic evaluation of the 45.5 kb of genomic sequence suggests that a transcript is embedded in the region between the two *HNF4A* promoters, and that this transcript and *HNF4A* are transcribed in opposite directions from opposing strands. We verified the expression of the embedded transcript in HepG2 human hepatoma cells by RT-PCR; however, we were unable to experimentally verify the direction of transcription in those cells. In an effort to isolate and characterize the novel transcript, we screened a human liver cDNA library using a 218 bp genomic DNA probe specific for a predicted exon of the transcript. We identified a 564 bp transcript, which contains two transcribed transposable elements, has no polyadenylation signal, has no open reading frame,is continuous on the genomic DNA, and is not homologous to any known protein coding sequence. These features are strikingly similar to the characteristics of noncoding RNAs, particularly antisense RNA. Secondary structure predicitions also suggest that the embedded gene resembles a noncoding RNA. In addition, the novel transcript and *HNF4A* are transcribed in opposite directions from opposite DNA strands. Gene profiling of the novel transcript and *HNF4A* indicate that they are expressed in many of the same tissues (kidney, liver, fetal liver, colon, small intestine, and adrenal gland), some of which are important in the development and progression of T2DM. The location and direction of transcription of this possible noncoding RNA suggests that it may play a role in regulating *HNF4A* gene expression. Further experiments are underway to fully characterize the transcript and investigate its involvement in the regulation of *HNF4A* expression.

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Characterization of the Region of 4q Associated with Hereditary Benign Intraepithelial Dyskeratosis. *B. Layfield[1], W. Lambert[1], M. Bembe[1], J. Clapp[2], J. Hewitt[2], K. Bastress[1], R. Lemmers[3], S. van der Maarel[3], R. Allingham[4], M. Hauser[1,4].* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Institute of Genetics, Queens Medical Center, University of Nottingham, United Kingdom; 3) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 4) Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Hereditary benign intraepithelial dyskeratosis (HBID) is an autosomal dominant cell proliferation disorder characterized by opaque epithelial plaques of the conjunctiva and oral mucosa. This disorder has been linked to a near-telomeric DNA duplication on 4q that includes a partial duplication of the D4Z4 repeat. The D4Z4 region has been reported to be a transcriptional silencer. We used real time PCR to analyze the expression of 19 genes near or within the HBID duplication in conjunctival cell lines and tissue. First strand cDNA was synthesized from primary cell lines from a HBID ocular lesion and control conjunctiva. No consistent pattern of expression as a result of the duplication was observed. One of the 4q genes analyzed was FAT, a member of the cadherin superfamily that functions in cell-cell adhesions, and whose expression level was decreased in HBID patients. FAT is an attractive candidate for the causative HBID gene: it is expressed in ocular tissues, and mutations in the fat locus in Drosophila cause defects in epithelial cell proliferation control and morphogenesis. Transcriptional silencing of the copy of the FAT gene located on the chromosome carrying the duplication, followed by somatic mutation of the second FAT allele in the conjunctival epithelium of HBID patients could give rise to abnormal cell-cell junctions and/or communication, thus leading to uncontrolled cell proliferation. To test this potential two-hit mechanism, we sequenced the FAT gene in DNA from peripheral blood from a HBID patient and from a cell line generated from lesion tissue removed from the same patient. No novel lesion-specific somatic mutations were observed, suggesting the hypothesis that FAT is the causative HBID gene via a two-hit mechanism is not valid.

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Mannose binding lectin haplotypes are not associated with recurrent spontaneous abortion. *D. Berger[1], W. Hogge[2], R. Ferrell[1].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Ob/Gyn, Univ Pittsburgh, Pittsburgh, PA.

Recurrent spontaneous abortion (RSA) affects up to 5% of all pregnancies and is likely to be of multifactoral etiology, with both genes and environment contributing to risk. Several infectious agents are known to cause late term pregnancy loss and an infectious etiology for RSA has been proposed. Mannose binding lectin (MBL) is a C-type lectin that plays an important role in innate immunity and low plasma levels of MBL are a known cause of severe infantile infection. Plasma levels of MBL are largely genetically determined and can be predicted by examining single nucleotide polymorphisms (SNPs) in the proximal promoter and exon 1 of the MBL2 gene. To test the hypothesis that MBL haplotypes leading to reduced levels of circulating MBL are significantly associated with idiopathic RSA, we conducted a case/control study of RSA. Cases (n=273) and controls (n=236) were drawn from the obstetrical population served by Magee-Womens Hospital, Pittsburgh, PA and the Center for Reproductive Medicine, Minneapolis, MN. RSA was defined as having two or more clinically recognized spontaneous abortions of unknown etiology prior to 22 weeks gestation. Subjects were genotyped for missense mutations in exon 1 (R52C, G54D and G57E) and functional promoter SNPs at -550 CG and -221 GC by standard methods. Allele frequencies were estimated by gene counting and compared to the expectations of Hardy-Weinberg equilibrium by chi square analysis. Haplotypes were estimated by maximum likelihood using the EM algorithm and single-locus tests. Allele and haplotype frequencies were compared in cases and controls using chi square contingency table analysis. Finally, subjects were classified by haplotypes based on predicted MBL levels (high, intermediate, low). No significant association was observed between MBL genotypes, haplotypes or predicted plasma MBL levels and increased genetic risk of recurrent pregnancy loss among women whose pregnancy loss is not associated with chromosomal abnormalities. Despite prior smaller studies reporting an association between RSA and variation at the MBL2 locus, we conclude that the MBL2 locus is not a significant determinant of risk of RSA.

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Regulation of Adrenergic Receptor $_2$ by C/EBP and cAMP Pathways. *T. Higgins, I. Tchivileva, C. Downey, W. Maixner, L. Diatchenko.* Neurosensory Disorders Unit, School of Dentistry, University of North Carolina, Chapel Hill, NC 27599.

Adrenergic receptor $_2$ (ADRB2) is a primary target for epinephrine. It plays a critical role in mediating physiological responses to environmental stressors and affects a complex array of psychological and physiological characteristics: including psychological distress, blood pressure and pain sensitivity. However, on immune cells ADRB2 functions as an inflammatory mediator. Increased pain sensitivity is one of the characteristics of both acute and chronic inflammation. We thus hypothesized that regulation of ADRB2 expression under inflammatory conditions would mediate inflammation-induced pain. We first searched the ADRB2 promoter region for putative binding sites of pro-inflammatory mediators, which revealed CREB (cAMP response element binding protein) and C/EBP (CCAAT/enhancer binding protein) binding sites. We constructed a luciferase reporter vector containing three major polymorphic variants haplotypes) of a 1.9kb human ADRB2 promoter region, and transiently transected the constructs into neuronal and immune cell lines. These variants showed different basal activity in all assessed cell lines. We measured promoter response to various treatments; modulating inflammatory state. Cells were stimulated with epinephrine and forskolin and co-transfected with an expression vector containing C/EBP or C/EBP. In the neuronal cells, reporter activity decreased significantly below baseline upon stimulation, and increased upon co-transfection with C/EBP and C/EBP. Stimulation with forskolin in the immune cells increased reporter activity. In summary, we demonstrated; i) different levels of basal promoter activity related to three major human haplotypes; ii) tissue specific regulation of ADRB2 promoter at the basal level and upon treatment; iii) haplotypes exhibited similar response patterns, demonstrating haplotype-independent transcription regulation by C/EBP and cAMP. These results are the first to demonstrate regulation of ADRB2 by C/EBP and cAMP pathways, and suggest a mechanism by which inflammation modulates pain sensitivity. Supported by NIH grants DE016558 and NS045685.

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Efficient gene transfert into normal human B lymphocytes with the chimeric adenoviral vector AD5/F35. *D. Jung[1,2], S. Néron[1,2], M. Drouin[1], A. Jacques[1].* 1) Dept Research & Development, Hema Quebec, Sainte-Foy, PQ, Canada; 2) Department of Biochemistry and Microbiology,Laval University, Québec, PQ, Canada.

The failure to efficiently introduce genes into normal cells such as human B lymphocytes limits the characterization of their function on cellular growth, differentiation and survival. Recent studies have shown that a new adenoviral vector Ad5/F35 can efficiently transduce human haematopoietic CD34+ progenitor cells. In this study, we compared the gene transfer efficiencies of the Ad5/F35 vector to that of the parental vector Ad5 in human B lymphocytes. Peripheral blood B cells obtained from healthy individuals were cultured in vitro using CD40-CD154 system. Normal B lymphocytes were infected with replication-defectives Ad5 and Ad5/F35, both containing the GFP reporter gene, and transduction efficiencies were monitored by flow cytometry. Ad5 was highly ineffective, infecting only about 5 % of human B lymphocytes. In contrast, Ad5/F35 transduced up to 60% of human B lymphocytes and GFP expression could be detected for up to five days post infection. Importantly, physiology of B lymphocytes such as proliferation, viability and antibodies secretion were unaffected following Ad5/F35 transduction. Finally, we observed that memory B lymphocytes were more susceptible to Ad5/F35 infection than naïve B lymphocytes. Thus, our results demonstrate that the adenoviral vector Ad5/F35 is an efficient tool for the functional characterization of genes in B lymphopoiesis.

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Identification of novel exons and different isoforms for VSX1. *S.M. Hosseini*[1,2], *E. Heon*[1,2,3]. 1) Program in Genetics & Genomic Biology, The Hospital for Sick Children Research Institute, Toronto, Canada; 2) Institute of Medical Science, University of Toronto, Toronto, Canada; 3) Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Canada.

Background: VSX1 is a homeobox transcription factor important for terminal differentiation of the retina. Mutations in *VSX1* have been found in patients affected with two distinct types of corneal dystrophies: Posterior Polymorphous Corneal Dystrophy (PPCD) and Keratoconus. Both conditions are genetically heterogeneous. However, the family originally used for mapping of PPCD to chromosome 20, did not show any coding sequence change in *VSX1*. Putative disease causing mechanisms include either the involvement of unsequenced regulatory sequences, uncharacterized isoforms, splicing defects or the involvement of a neighboring gene.

Purpose: To investigate alternative splicing events in *VSX1* and to characterize the possible isoforms of VSX1.

Methods: ECgene v1.2 gene prediction was used for exon prediction. Total RNA was extracted from the human retina using a phenol-chloroform based method (TRIzol). 3 RACE (Rapid Amplification of cDNA Ends) was performed on human retinal RNA using two different gene specific primers in the known exons 2 and 4. Second round of PCR were performed using nested primers. PCR product was run on either 4% polyacrylamide gel or 1.2% agarose gel. All observed bands were cut, gel purified, cloned into pGEM-T vector and sequenced.

Results: We identified novel downstream exons and characterized at least 8 different isoforms with the evidence of alternative splicing events.

Conclusion: Different isoforms of proteins are known to be associated with disease causing mutations in other diseases such as Retinitis Pigmentosa and Usher syndrome. Our data shows the existence of multiple VSX1 isoforms and warrants further investigation into their potential pathologic role in PPCD.

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5 triphosphate of *in vitro* transcribed siRNAs induce antiviral response in cell culture. *M. Grippo[1], T. Campos-Pereira[1], V. B. P. Pascoal[1], P. A. R. Aguiar[1], M. S. Silva[1], R. Gilioli[2], I. Lopes-Cendes[1].* 1) Medical Genetics, UNICAMP/FAC CIENCIAS MED, Campinas, Brazil; 2) Laboratory of Animal Quality Control, CEMIB,UNICAMP, Campinas, Brazil.

RNA interference(RNAi)is a technique for specific and gene silencing by double stranded RNA molecules(dsRNAs).The objective of this study was verify the efficiency of this new technique in the combat of the MHV-3 virus (hepatitis murine type 3).MHV-3 is the most frequently found pathogen in laboratory mice. It is an enveloped virus with a large plus-stranded RNA genome belonging to the Coronaviridae family and codifies structural proteins such as: S(spike); M(transmembrane) ,E(membrane)and N (nucleocapsid) .The N protein was chosen as the target for siRNA mediated silencing in our experiments.Design of the siRNA molecules was based on the MHV-3 genome sequence available(GenBank AF 201929).We obtained 2 siRNA molecules,named 634 and 910, based on RNA messenger position. Molecules were produced by in vitro transcription through a T7 RNA polymerase.We used L-929 cell culture placed in 24 wells plates and 270 ng/well of each siRNA molecule, separately, in each experiment. Cultured cells were transfected with lipofectin.After transfection, cells were infected with 1 TCID50 of the MVV-3 virus, and cultures were observed up to 48 hours. We found that siRNAs synthetized with T7 RNA polymerase system can trigger a potent induction of IFN in L929 cell.This effect was subsequently eliminated by siRNA treatment with calf intestine phosphatase prior to transfection. In addition, we observed a significant reduction of the citopatic effect of the MHV-3 virus in cell cultures treated with siRNA 910 but not with siRNA 634.In conclusion, we were able to demonstrate a potential therapeutic application for iRNA in the treatment of *in vitro* MHV-3 viral infection. In addition, our data show that although cost-effective and efficient, *in vitro* transcribed siRNAs must be dephosphorylated before *in vitro* use.

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The quantification of the allelic variations of gene expression by real-time TaqMan PCR. *K. Kobayashi[1], A. Suzuki[1], Y. Kochi[1], R. Yamada[2,1], K. Yamamoto[1,3].* 1) Laboratory for Rheumatic Diseases, SNP Research Center, RIKEN, Yokohama, Japan; 2) Center for Genomic Medicine, Kyoto University, Kyoto, Japan; 3) Dept. of Allergy and Rheumatology, University of Tokyo, Tokyo, Japan.

Recently, multiple studies have shown that human single nucleotide polymorphisms (SNPs) affect gene regulation, resulting allelic imbalance of gene expression. The finding of these regulatory SNPs leads to understanding phenotypic diversity and the identification of alleles that modify disease risks. Therefore, it is important to quantify the allelic difference of gene expression in vivo to identify regulatory SNPs. We applied TaqMan assay on cDNA from heterozygous cells to quantify transcripts in allele-specific manner. We validated applicability of the method as follow. Initially the calibration curve was obtained by assaying mixture of genomic DNA samples from known homozygous individuals with various mixture ratio ranging from 4:1 to 1:4. Then we estimated mixture ratio of genomic DNA samples from known SNP genotypes including heterozygozes. After correction with the calibration curve, the estimates were corresponded well with the given ratio. After validation of the method, we examined allele-specific gene expression in peptidylarginine deiminase 4 (PADI4) and Fc receptor-like (FCRL3) genes, which were reported to be associated with rheumatoid arthritis (RA), and of which allelic imbalances of gene expression were observed. We used TaqMan probes, rs11203366 (G/A) and rs945635 (G/C) as markers, located in exonic region of PADI4 and FCRL3, respectively. We measured the signal intensity using cDNA and DNA which were isolated from peripheral blood leukocytes of healthy heterozygous individuals, and we calculated allele-specific gene expression ratio. We showed that RA susceptible allele-specific PADI4 expression mean ratio '} SD was 1.21 '} 0.33 in 7 heterozygous individuals, and RA susceptible allele-specific FCRL3 expression mean ratio '} SD was 1.99 '} 0.50 in 5 heterozygous individuals. These data supported the hypothesis that expression levels of PADI4 and FCRL3 are associated with RA susceptibility[1, 2]. 1) Nat. Genet. 2003, 34:395-402, 2) Nat. Genet. 2005, 37:478-485.

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Real-time PCR as a tool in the identification of functional oncology target genes. *S. Rhodes, P. Culp, E. Tom, S. Tanlimco, W. Schneider, A. Sharp, H. Li, T. Anderson, R. DuBridge.* Protein Design Labs, Fremont, CA.

For RNAi assays to give a clear indication of the functional activity of an oncology target gene, a reduction in protein and mRNA levels upon siRNA treatment must be seen to correlate with a reduction in the growth rate of cells expressing the target. At the same time siRNA treatment of cells not expressing the target should have no effect on tumor cell growth. This requires an accurate knowledge of the expression levels of the target gene in each cell line in the study. Expression data from a wide variety of human tumor cell lines was accumulated using proprietary custom designed Genechips (Hu03) made by Affymetrix . We used Taqman real-time PCR for selected oncology targets in these cell lines to determine absolute expression levels. This allowed positive and negative cell lines to be chosen for cell growth assays using three different siRNAs (Dharmacon) directed against each target gene. We found it necessary to design our taqman assays such that they measured sequences 5 to the siRNA cleavage site to generate an accurate assay for mRNA down modulation. SiRNAs, which showed an apparent reduction in growth in at least some of the positive cell lines, but no effect in the negative cell lines, were assumed to be specific. These transfectants were then screened by FACS for protein down-modulation and/or real-time PCR for mRNA down-modulation. Functional targets were then defined as those genes that showed specific growth effects that were proportional to their degree of down modulation.

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Knockdown of SMN expression in NSC34 motor neuron cell line: Insights into the pathogenesis of spinal muscular atrophy (SMA). *M. Ganta, S.M. Grzeschik, C.H. Wang.* Dept Neurology, Stanford Univ, Stanford, CA.

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease affecting approximately 1/10,000 live births. SMA is characterized by degeneration of spinal cord motor neurons and muscular atrophy. SMA is caused by decreased levels of the survival of motor neuron (SMN) protein as a result of mutations in the SMN1 gene. Although SMN is ubiquitously expressed in all cells, motor neurons are selectively vulnerable to decreased amounts of SMN. The reason for the selective vulnerability of motor neurons in SMA is still unclear. Recently it has been suggested that SMN may play a role in neurite outgrowth and formation and maintenance of neuromuscular junctions. To gain a further understanding into the role of SMN in these motor neuron functions, we used RNA interference (RNAi) to knockdown SMN expression in immortalized mouse motor neuron cells (NSC34 cells). NSC34, a hybrid spinal cord motor neuron-neuroblastoma cell line, possesses properties of primary motor neurons, including generation of action potentials, acetylcholine production, expression of neurofilament proteins, and innervation of myotubes in vitro. A shRNA (short hairpin RNA) targeting SMN was cloned into a plasmid expression vector also expressing coral GFP (cGFP). Expression vector without the shRNA targeting SMN was used as a control. NSC34 cells were transiently transfected using Lipofectamine 2000 reagent. Cells were fixed at 24, 48, 72, 96 and 120h after transfection and stained with a monoclonal antibody against SMN. At least 15 cells in each group and at each time point were visualized by confocal microscopy. All cGFP positive cells transfected with plasmid containing shRNA targeting SMN showed a decrease in the intensity of SMN staining, indicating a knockdown in SMN levels, compared to controls and untransfected cells. Reduction in levels of SMN was observed as early as 72h and extended up to 120h post transfection. In conclusion, we have created a novel cell culture model to study the function of SMN in a relevant cell type.

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Retinal Dystrophin isoform Dp260 expression is induced by steroids and the up-regulation is likely mediated by glucocorticoid receptors (GRs) and two glucocorticoid response elements (GREs) in an internal promoter of the Duchenne (DMD) gene. *D.M. Pillers, B. Malmin, J.S. Pang.* Dept Pediatrics,Ophthalmology, and Molecular & Medical Genetics, Oregon Health & Sci Univ, Portland, OR.

Background: Duchenne muscular dystrophy (DMD) is an X-linked disorder leading to severe myopathy and early death. The DMD gene encodes dystrophin, which in muscle, is expressed as a 427 kDa protein. Non-muscle manifestations include abnormal electroretinograms (ERGs) and defects in cognition. Dp260 is the predominant isoform expressed in retina, and is implicated in normal retinal electrophysiology. Objective: To determine how Dp260 expression is regulated, and whether manipulation of the regulatory elements could lead to expression of Dp260 in muscle or retina. Methods: A human genomic fragment from a cosmid containing the promoter of Dp260 was subcloned into the pGL2 luciferase reporter vector. The vector was transfected into cell lines (Y79, C2, HeLa) and exposed to methylprednisolone. Site-directed mutagenesis was used to determine the contributions of elements in the promoter region. A gel shift assay was performed using HeLa cell extract and a super-shift using anti-GR. A potential role of a glucocorticoid receptor (GR) was investigated by transfection into the COS-7 GR-defective cell line, and by exposure to the glucocorticoid antagonist, RU486. Results: Sequence analysis identified two glucocorticoid response elements (GREs. Both elements are important in the steroid response, but the proximal GRE has the greatest effect on gene expression. The importance of GRs in mediating the response was demonstrated by a lack of stimulation of Dp260 expression in the COS-7 cell, by a super-shift in the presence of anti-GR, and by the ability of the glucocorticoid antagonist, RU486, to block the response. Conclusions: Upregulation of Dp260 expression by methylprednisolone is mediated by the interaction of DNA-binding GR proteins with GREs in this internal promoter region of DMD. Use of the GR/GRE system by Dp260 is unique for genes expressed in retina. Novel opportunities to manipulate dystrophin expression in vivo may lead to new pharmacogenetic approaches to the therapy of DMD.

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The Extent and Functional Consequences of Mutation at the *PRODH* locus. *A.S. Willis[1,2], H.U. Bender[1,2], G. Steel[1,2], A. Pulver[3], D. Valle[1,2].* 1) Institute of Genetic Medicine, Johns Hopkins Univ School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute, Johns Hopkins Univ School of Medicine, Baltimore, MD; 3) Dept Psychiatry, Johns Hopkins Univ School of Medicine, Baltimore, MD.

PRODH maps to 22q11 within the critical region for 22q11 deletion syndrome and encodes proline oxidase (POX), a mitochondria inner membrane enzyme that catalyzes the first step in proline catabolism. Severe loss of function mutations in *PRODH* cause Type I hyperprolinemia while mild mutations have been implicated as possible susceptibility alleles for schizophrenia. Additionally, alterations in the level of POX expression have been shown to influence apoptosis and to play a role in certain cancers.

To better understand the extent and functional consequences of *PRODH* mutations, we determined the exonic and flanking intronic sequences of *PRODH* in a group of 25 individuals with 22q11 deletion syndrome including several with hyperprolinemia. We found 12 missense and 7 synonymous mutations in the coding exons as well as 2 - 5UTR and 51 intronic variations. We have recently reported the functional consequences of 9 of the missense mutations and are in the process of testing the remainder (Bender et al, *Am J Hum Genet* 76:409-420, 2005).

Additionally, to better understand the pathological mechanisms of the *PRODH* missense mutations, we conducted studies to determine the oligomerization state of POX. We produced cDNA clones of *PRODH* tagged with C-terminal HA or myc as well as analogous cDNA clones for hydroxyproline oxidase (HPOX), which shares 31% amino acid identity with POX. Co-immunoprecipitation experiments showed that POX subunits can oligomerize with themselves or with HPOX subunits. Studies are in progress to determine the stoichiometry of and domains involved in the oligomerization and the functional consequences of variation on the homo- or hetero-oligomerization of POX.

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TAB2, TRAF6 and TAK1 mediate NF-kB activation induced by the TNF-receptor Edar. *A. Morlon, A. Munnich, A. Smahi.* INSERM U393, Hopital Necker, France.

Activation of NF-kB by the TNF receptor Edar and its downstream adaptator Edaradd is essential for the development of hair follicles, teeth, exocrine glands and other ectodermal derivatives. Dysfunction of the Edar signalling pathway cause hypohidrotic/anhidrotic ectodermal dysplasia, a disorder characterized by sparse hair, lack of sweat glands and malformation of teeth. By virtue of a two-hybrid screening, we isolated TAB2 as a binding partner of Edaradd. Here we show that TAB2 and also TRAF6 and TAK1, two partners of TAB2 in IL-1/TLR and RANK signalling, coimmunoprecipitate with Edaradd in mammalian cells. Moreover, our results show that dominant-negative forms of TAB2, TRAF6 and TAK1 inhibit Edaradd-induced NF-kB activation in 293 cells. These results support the involvement of the TAB2/TRAF6/TAK1 complex in Edar signalling. These findings have important implications for our understanding of NF-kB activation pathways and pathophysiology of ectodermal dysplasia.

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The Mechanism of degenerative muscle defects in myotonic dystrophy. *P.S. Sarkar, T. Ashizawa, R. Gao, W. Xu.*
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Myotonic dystrophy type1 (DM1) is characterized by the dysfunction of the muscle structure and function (1). Delayed muscle differentiation and hypotonia are the predominant features in congenital DM1 whereas myotonia, atrophy and weakness are the features in adult onset DM1 (1). DM1 genetic mutation is the expansion of CTG repeat in the 3 UTR of DMPK on chromosome 19q13.3 and massive expansion is associated with dystrophic muscle defects(1). The mechanism by expanded CTG repeat causes dystrophic muscle defects is not established. Transgenic mice expressing ~250 units of CTG repeats in skeletal muscle develop myotonia and structural defects reminiscent of DM1 (2). Sequestration of muscle-blind proteins by expanded CUG RNA was found to be the pathogenic mechanism in the CUG mice (3). Subsequent studies with muscle-blind (Mbnl1) knockout mice confirmed that the loss of muscle-blind function is one of the important mechanistic steps required to cause myotonia (2,3). Neither Mbnl1 knockout nor the CUG transgenic mice develop dystrophic muscle defects, hallmark DM1 features(4). Our goal is to understand the mechanism by which massive expansion of CTG repeats causes dystrophic muscle defects in DM1. Our ability to clone longer repeats allows us the advantage of not only in developing appropriate mouse models but also identifying a novel zinc-finger protein, sequestered by expanded CUG RNA. Our studies show that the novel protein is sequestered in nuclei and co-localizes with CUG-RNA. We will present evidences to show that multiple proteins are sequestered by expanded CUG RNA and sequestration of proteins in addition to muscle-blind proteins is the major pathogenic mechanism underlying the complex dystrophic muscle defects DM1. 1)Harper PS. (2001) Myotonic Dystrophy, 3nd edn. Saunders, London 2) Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, et al. (2000)Science 289, 1769-1773. 3) Mankodi A, Urbinati CR, Yuan QP, Moxley RT, Sansone V, Krym M, Henderson D, Schalling M, Swanson MS, Thornton CA. (2001) Hum Mol Genet 10: 2165-70 4) Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, et al. (2003) Science 302, 1978-1980.

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Genetic Analysis of Nonstop mRNA Decay in *Caenorhabditis elegans*. *J. PARVAZ, C. SCHARTNER, P. ANDERSON.* DEPARTMENT OF GENETICS, UNIVERSITY OF WISCONSIN-MADISON, MADISON, WI.

Degradation of messenger RNAs (mRNAs) is a fundamental aspect of gene expression. Nonstop decay (NSD) is an mRNA degradation pathway that selectively degrades mRNAs lacking an in-frame stop codon. NSD occurs in the cytoplasm of yeast and human cells and requires active protein synthesis. NSD in yeast is distinct from the major pathways of mRNA turnover, because it functions in the absence of factors required for the deadenylation-dependent 5' decay pathway or for nonsense-mediated mRNA decay (1). Instead, yeast NSD occurs via an exosome-dependent 3' pathway (2). The mechanisms of NSD in other organisms are not well understood.

We present evidence that NSD exists in the nematode, *Caenorhabditis elegans*. The steady-state abundance of mRNAs expressed from a nonstop reporter gene, is greatly reduced relative to controls containing an in-frame stop codon. We have mutagenized worms expressing a nonstop reporter mRNA, and identified seven mutants that elevate reporter gene expression. We are investigating these mutants both genetically and molecularly to determine whether they encode factors needed for *C. elegans* nonstop decay.

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(2) van Hoof A, Frischmeyer PA, Dietz HC, Parker R, *Science* 295, 2262 (2002).

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Analysis of conserved regulatory elements in the glucocerebrosidase gene locus. *K.S. Hruska[1], M.E. LaMarca[1], S.G. Ziegler[1], M.E. Portnoy[2], NISC Comparative Sequencing Program[2], E.D. Green[2], E. Sidransky[1].* 1) Section on Molecular Neurogenetics, NIMH and Medical Genetics Branch, NHGRI; 2) Genome Technology Branch, NHGRI; NIH, Bethesda, MD.

Delineating the mechanisms that coordinate gene expression has become one of the primary goals of the post-genomic era of biology. Regulatory elements in the promoter and first exon of the human glucocerebrosidase gene (GBA) have been reported previously, but the availability of sequenced genomes has simplified functional analyses and greatly expanded their scope. The comparison of orthologous sequences from multiple species in parallel can be used to detect evolutionarily conserved regions that may reflect functional elements. MultiPipMaker alignments of the 58.5 kb genomic region comprising the genes clorf2, GBA, metaxin 1 (MTX1) and thrombospondin 3 (THBS3) from human and ten mammals demonstrated strong conservation of exons in all four genes, especially THSB3. Therefore, we performed a WebMCS analysis focused on mammalian sequences aligning with the 39.4 kb segment of human genomic sequence that encompasses MTX1, GBA and their respective pseudogenes. At the 95% threshold, 46 multi-species conserved sequences (MCSs) were detected with an average length of 43 bp. Two MCSs were identified in the 3'-UTR region of GBA that did not correspond to exonic sequence of the metaxin pseudogene; one 25-bp MCS represents the THBS3 enhancer previously described within intron 6 of MTX1, while a second 25-bp MCS appears to be novel. No MCSs were identified in the 4 kb region upstream of GBA. When the aligned multi-species sequences were inspected for consensus regulatory motifs using the Genomatix Suite applications, however, a shared framework of transcription factor binding sites was identified upstream of GBA in human, chimp, galago, cow, dog and fruit bat. This potential regulatory module was not detected in murine GBA or human GBA pseudogene (psGBA) sequences. The regions containing these conserved motifs are being tested *in vitro* for their effects on the transcriptional regulation of GBA.

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Molecular characterisation of the aminopeptidase P gene regulatory region. *A.L. Jones, A.M. Carter, P.J. Grant, N.M. Hooper.* The LIGHT Laboratories, University of Leeds, Leeds, UK.

Aminopeptidase P (APP) degrades des-arg9-bradykinin and decreased APP has been linked to ACE inhibitor-induced angioedema. We hypothesised polymorphisms in the APP 5 regulatory region influence APP expression. The aims of this study were to (1) characterise 3kb of the 5 regulatory region of APP (2) identify novel polymorphisms (3) determine the contribution of polymorphisms to variance in APP in the Leeds Family Study (4) determine the influence of polymorphisms on reporter gene expression. Key regulatory regions were identified by ligating 3kb of APP regulatory region upstream of the luciferase gene in pGL3 and generating nested deletion constructs using Erase-a-Base. Luciferase was analysed in HepG2 cells transiently transfected with the nested deletion constructs. The Transgenomic WAVE system was used to identify polymorphisms. The polymorphisms were genotyped in the family study (537 individuals) and plasma AP-P activity was measured using the substrate Lys(Abz)-Pro-Pro-pNA. The influence of polymorphisms on luciferase activity was determined by site directed mutagenesis of the APP-pGL3 construct and transient transfection of HepG2s. Statistical analyses were carried out using SPSS and SOLAR. Nested deletion analysis identified a putative enhancer region between -2310bp and -2046bp and a minimal promoter located in the first 146bp upstream of the +1 site. Three common SNPs (-201GA, -1512GT, -2207CA) and 2 rare (+27CT, -1078GA) were identified. The -201GA and -2207CA SNPs were significantly associated with plasma AP-P activity (-201GA: GG=1.97 [1.76, 1.98]; GA=2.27 [1.98, 2.60]; AA=2.32 [1.96, 2.74] mol/ml/min, p0.001) and (-2207CA: CC=2.16 [2.04, 2.29]; CA=1.75 [1.57, 1.95]; AA=1.06 [0.89, 1.26] mol/ml/min, p0.001) and SOLAR analyses indicated that the -2207CA and -201GA SNPs contributed to 7.36% and 1.43% of the phenotypic variation in AP-P respectively (age and sex adjusted). These associations were supported by luciferase reporter gene analyses. These data suggest that both the -2207CA and -201GA polymorphisms, which are located in the vicinity of the 2 key regulatory regions, are functional and contribute to variance in plasma APP levels.

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Transcriptional repression of WEE1 by Kruppel-like factor 2 is involved in DNA damage-induced apoptosis. *F. Wang[1], Y. Zhu[1], Y. Huang[1], S. McAvoy[1], W.B. Johnson[2], T.H. Cheung[3], T.K. Chung[3], K.W. Lo[3], S.F. Yim[3], M.M. Yu[4], H.Y. Ngan[3], Y.F. Wong[3], D.I. Smith[1].* 1) Division of Experimental Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA; 2) Departments of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN, USA; 3) Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, People's Republic of China; 4) Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, People's Republic of China.

Human Kruppel-like factor 2 (KLF2) is a Cys2/His2 zinc-finger-containing transcriptional factor, which is involved in multiple cellular pathways. Utilizing gene expression profiling to identify aberrantly expressed genes in ovarian cancer, we found that KLF2 was significantly and specifically downregulated in ovarian tumors. After reintroducing KLF2 into ovarian cancer cell lines, we observed decreased cell growth and increased sensitivity to DNA damage-induced apoptosis. Analysis of genes that could be potential targets of KLF2 revealed that KLF2 negatively regulated WEE1 expression. WEE1 encodes a tyrosine kinase that regulates the G2/M cell cycle transition. Expression of KLF2 markedly repressed the transcription of WEE1 by directly binding to an SP1/CPBP motif located between -252 bp and the start codon of the WEE1 promoter. Both activation and zinc-finger domains of KLF2 were required for this suppression of Wee1 expression. In addition, we demonstrated that Wee1 expression prevents cancer cells from undergoing apoptosis in response to DNA damage; however, this resistance was abolished by coexpression of KLF2, which inhibits WEE1 transcription. Thus, the level of WEE1 is regulated by KLF2 and enhanced KLF2 expression sensitizes cells to DNA damage-induced apoptosis.

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SUMOylation of the SOX10 transcription factor modulates its transcriptional activity with different functional consequences. *M. Girard, M. Goossens.* Dept Genetics, INSERM U654, Creteil, France.

SOX10 is a transcription factor of the SOX family that is expressed in the neural crest by the time of its emergence, and then in neural crest derivatives forming sensory, sympathetic and enteric ganglia, melanocytes and glial cells of the nervous system. Heterozygous SOX10 mutations cause Waardenburg syndrome type IV (WS4), associating Hirschsprung disease (intestinal aganglionosis) and Waardenburg syndrome (pigmentation defects and sensorineural deafness). Some WS4 patients also present with a neurological phenotype, leading to PCWH syndrome (Peripheral neuropathy, Central leukodystrophy, Waardenburg syndrome and Hirschsprung disease). A two-hybrid screen revealed an unsuspected aspect of SOX10 regulation: sumoylation. Sumoylation is a highly dynamic post-translational modification consisting of attachment of a small peptide, SUMO, to a target protein. This modification has various functional consequences on target proteins and affects stability, activity and localisation of specific transcription factors. Sumoylation occurs on a KXE consensus sequence where indicates a hydrophobic amino acid, K is the lysine of attachment of SUMO and E is a glutamate. The SOX10 protein has three potential SUMO consensus sites, all of them are functional and lead to SOX10 sumoylation in vivo. Sumoylation does not affect SOX10 sub-cellular localisation, but modulates its transcriptional activity. Indeed, mutation of the three SOX10 sumoylation sites significantly increases its transcriptional activation of two of its target genes, GJB1 (encoding connexin 32) and MITF. Interestingly, sumoylation of SOX10 appears to have different consequences on its function depending on the site that is used for SUMO attachment. Sumoylation modulates SOX10 protein interactions, including its own dimerization and interaction with its cofactors EGR2 and PAX3. Sumoylation is the first post-translational modification described for SOX10 and leads to a fine regulation of its function, in agreement with the multiple roles of this factor. This work allows a better understanding of SOX10 function in development and enlightens a new aspect of its regulation.

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A 300 bp of Mouse *Col10a1* Distal Promoter Element is Sufficient to Mediate its High-level Tissue-specific Expression in Hypertrophic Chondrocytes *In Vivo*. *Q. Zheng[1], B. Keller[1], G. Zhou[1], D. Napierala[1, 2], Y. Chen[2], A. Parker[3], B. Lee[1, 2].* 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) Howard Hughes Med Inst. Houston, TX; 3) Respiratory and Inflammation Res. Area, AstraZeneca , Cheshire U.K.

Type X collagen gene (*Col10a1*) is specifically expressed in hypertrophic chondrocytes. Identification of the cis-regulatory element and its binding factors for tissue-specific mouse *Col10a1* expression is essential for understanding the mechanisms that specify endochondral ossification and underlie the molecular pathogenesis of skeletal dysplasia. We have previously reported that 4kb *Col10a1* promoter containing Runx2 elements can specifically drive weak reporter gene (*LacZ*) expression in the lower hypertrophic zone of transgenic mice. Here we report generation of additional transgenic mouse lines harboring various *Col10a1* promoter/intronic fragment (Tg-10 kb) or distal *Col10a1* element (Tg-6kb and Tg-4.6kb) driving *LacZ* as reporter. High-level tissue-specific reporter expression was observed in these transgenic mice. To clarify the contribution of this *Col10a1* distal element to its tissue-specific expression, transgenic mouse lines using two copies of the 700 bp putative enhancer element (-4.6 to -3.9 kb) upstream of the *Col10a1* basal promoter driving *LacZ* as reporter (Tg-2xEnh) have been established and showed similar tissue-specific expression pattern. More recently, we have generated transgenic founder mice carrying reporter construct containing four copies of highly conserved 300 bp fragments within this region (-4.3 kb to -4.0 kb) upstream of the same *Col10a1* basal promoter. These Tg-4x300 founder mice show similar hypertrophic chondrocyte-specific expression pattern to that of the Tg-4.6 kb, Tg-6 kb, Tg-10 kb and Tg-2xEnh mice. These data together suggest that this 300 bp highly conserved element contains cis elements sufficient for tissue-specific *Col10a1*/reporter expression throughout hypertrophic zone. Further dissecting of the cis regulatory element within this region and its binding factors will ultimately help to identify signaling pathways relevant to skeletal development, especially chondrocyte maturation.

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An SVA element in the 3UTR of the fukutin gene impairs transcriptional elongation in Fukuyama-type congenital muscular dystrophy. *F. Wang[1], Y. Nagai[1], H. Kurahashi[1,2], K. Kobayashi[1], H. Kano[1], T. Toda[1].* 1) Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan; 2) Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan.

Fukuyama-type congenital muscular dystrophy (FCMD) is a severe autosomal recessive muscular dystrophy accompanied by brain malformation. We previously isolated the responsible gene for FCMD, fukutin, through positional cloning, and found that 87% of FCMD chromosomes contain a 3 kb SVA-type retrotransposal insertion in the 3UTR of the fukutin gene. We also showed that the fukutin mRNA is greatly reduced in patient lymphoblasts, skeletal muscle and brain. However, the molecular mechanisms by which the SVA insertion influences fukutin mRNA expression have not been elucidated. In this study, we confirm that the retrotransposal insertion significantly decreases fukutin mRNA expression both in transiently transfected cells and in patient fibroblast culture. Analysis of mRNA stability using actinomycin D shows only a slight shortening of the half-life of fukutin mRNA by the insertion. On the other hand, induction of fukutin mRNA expression is greatly impaired by the insertion in an inducible cell line, suggesting transcriptional disruption. Chromatin immunoprecipitation analyses reveal that histone modifications in the promoter region of fukutin is not affected by the insertion in the fibroblasts. Further analysis of transcription by the nuclear run-on assay shows that transcriptional elongation is impaired by the insertion whereas transcriptional initiation is unaffected. We conclude that the SVA element in the 3UTR of the fukutin gene impairs transcriptional elongation. As SVA elements are thought to be currently active in the human genome, the SVA element inserted in the 3UTR of the fukutin gene may function through epigenetic mechanisms to down-regulate the host gene expression.

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A conserved splicing enhancer identified by alignment of intronic sequences. *I. Aznarez[1,2], D. He[1,2], G. Deng[1], J. Cheung[1], L.-C. Tsui[1,2,3], J. Zielenski[1], J. Parkinson[1,2], B.J. Blencowe[4].* 1) Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Molecular & Medical Genetics, University of Toronto, ON, Canada; 3) University of Hong Kong, Hong Kong; 4) Banting and Best, University of Toronto, ON, Canada.

Splicing requires accurate selection of splice sites (ss) promoted by elements that are bound by splicing factors. Intronic splicing enhancers in particular are not well characterized. Using CFTR as a starting point we performed multi-species alignments of genomic sequences. Intronic sequences showing high conservation near exon boundaries were considered as candidate splicing elements. Results showed that the intron sequences downstream of the 5'ss of 12 of the 26 CFTR introns contained conserved segments of 20-40 nucleotides. We screened these conserved segments for motifs and 9 out of 12 contained T-rich hexamers (5Ts/6). Conversely, T-rich motifs were found in only 2 out of 14 non-conserved sequences (p=0.004). To assess whether the T-rich motifs were present in other genes proximal to 5'ss, we scanned 26,555 protein-coding, intron-containing genes. Results showed a significant increase of T-rich motifs compared to compiled intronic nucleotide content (p=0.015). Pools of intronic sequences downstream of alternative and constant exons were analyzed independently, revealing a higher incidence of T-rich motifs downstream of alternatively spliced exons. To assess the distribution of T-rich hexamers, 1000 bp from +1 of all introns were analyzed revealing a cluster within the first 20 bp of intronic sequences starting at position +5. In addition, sequences downstream of pseudo 5ss (n=78) located in all exons and the first 150 nucleotides of each intron of CFTR were scanned for T-rich motifs. Results showed that these motifs occur less frequently downstream of pseudo 5ss (p=0.04). A candidate splicing factor, T-lymphocyte inducer of apoptosis (TIA-1) that promotes 5'ss selection, may be involved in the recognition of the identified T-rich motif based on previous analyses of three independent genes. Altogether, these results suggest that the T-rich motifs could function as an intronic splicing enhancer.

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Expression of Normal and Abnormal CPSI Transcripts Using a Eucaryotic-Replicating-CPSI containing BAC Expression System. *A. Eeds[1], L. Hall[1,3], R. Wade-Martins[2], A. Putnam[1], R. Price[1], D.. Mortlock[1], M. Summar[1,3].* 1) Center for Human Genetic Research, Vanderbilt Univ, Nashville, TN; 2) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 3) Dept of Pediatrics, Vanderbilt Univ, Nashville, TN.

RNA processing defect mutations are now known to be one ,if not the, most common causes of defective gene expression. We have found that 1/3 of the mutations in carbamyl phosphate synthetase I (CPSI) are of this nature. It has been difficult to study exact effects of these changes in patients without an expressing tissue source. We have previously reported the creation of a BAC expression system that replicates in eucaryotic cells and contains a selective agent. We have tested this construct containing a full copy of the CPSI gene and found that it produces normally spliced CPSI RNA. We have used homologous recombinant engineering to incorporate a mutation near the intron/exon boundary of intron 10, suspected of causing nonsense mediated decay, into this system. We have found that the transcript from this clone produces an abnormally spliced CPSI transcript with abnormal message levels compared to the control construct. This system provides a rapid and reliable method for assessing the affect of suspect RNA processing defects using a whole gene construct. This system can also be used to test the effects of mutations and polymorphisms on most aspects of a gene product and should prove to be a valuable tool in assaying functional significance.

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Human and mouse retinal alternative RNA splicing (AS) of a set of genes that are implicated in retinal degenerations. *F.Y. Demirci[1], B.W. Rigatti[1], M.B. Gorin[1, 2].* 1) Dept. of Ophthalmology, UPMC Eye Center, Ophthalmology and Visual Science Research Center, Univ. of Pittsburgh SOM, Pittsburgh, PA; 2) Dept. of Human Genetics, Univ. of Pittsburgh GSPH, Pittsburgh, PA.

Alternative RNA processing generates functionally diverse isoforms or provides a regulatory mechanism by modifying the elements that control RNA stability, localization, and translation. According to recent genome-wide analyses, ~75% of human and ~60% of mouse multi-exon genes are predicted to be alternatively spliced. The retina is among the top 3 tissues with the highest levels of tissue-specific AS and a number of human retinal disease genes are known to undergo AS. We used human and mouse databases to identify the known and/or predicted AS events for a set of retinal disease genes and we sought to experimentally confirm them as well as discover novel forms. Initially, 5 genes (*UNC119, RGR, NR2E3, NRL, CRB1*) were evaluated. Total RNA was isolated from retinas of 8-12 week-old mice and human retinal RNA was commercially obtained. The brain and spleen RNA samples were also obtained to determine the tissue-specificity of AS events. RT/PCR was performed to amplify the transcript fragments that are known or predicted to undergo AS. PCR fragments were gel-purified and directly sequenced. We identified some previously unreported retinal AS and alternative termination events in the set of transcripts that we investigated. Observed AS events included exon skipping, alternative exon inclusion, different splice site usage, and intron retention. Some of the murine variants were found to be different than the human variants, while others were confirmatory for the corresponding human forms. Conserved splicing events warrant particular consideration for their functional significance. AS appears to occur in retinal transcriptome more frequently and in a wider spectrum than previously thought. Identification and characterization of retinal transcript splice forms will guide further studies toward understanding the functional diversity created by these variants and/or their contribution to regulatory mechanisms, effect on retinal disease, and factors regulating retinal alternative RNA processing.

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Protein-protein interaction of the intracellular loops and NBD domains in CFTR. *S.J. Potts[1], M.J. McGinniss[2], F.M. Hantash[2], W. Sun[2], C. Strom[2].* 1) Advanced Diagnostics IT, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; 2) Genetic Testing Center, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Mutations in the human ATP-binding cassette (ABC) transporter, Cystic Fibrosis Transmembrane Regulator (CFTR), cause cystic fibrosis by either disruption of biosynthetic processing or by production of a functionally defective protein. From structures of other ABC transporters and biochemical experimental evidence, activity in each nucleotide binding domain (NBD) is coupled to transmembrane helices through two intracellular loops. Along with others, we have observed functionally impacting mutations in cystic fibrosis patients in both these intracellular loops and the putative areas of the NBDs that interact with these loops. With the recent crystal structure of NBD1 (Lewis, et al EMBO J 2004; 23:282-293) and a homology model for NBD2, further insight into the likely impact of these mutations on protein function is now possible. Using protein-protein docking software, ab initio modeling and threading algorithms (Simons et al. J Mol Biol 1997;268:209-225) for alignment with other solved protein structures, we are investigating the possible interactions of these loops with the two NBD domains in order to predict the functional significance of novel point mutations in CFTR. We include known biochemical assay data, sequence conservation across human CFTR orthologs and paralogs, and the available data on functional implications of mutations in these domains in other human ABC-transporters. From our predicted interaction of these loops with NBD domains we can restrict the potential topology arrangements of the transmembrane helices.

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ELOVL4 distribution in mouse, monkey and human eyes. *Y. Chen, D. Cameron, Z. Yang, G. Karan, K. Howes, Z. Tong, S. Hamaya, A. Azimi, Y. Zhao, K. Zhang.* Ophthalmology, University of Utah, Salt Lake City, UT.

Purpose: Stargardt-like macular dystrophy (STGD3) is an autosomal dominant form of early onset macular degeneration. The disease causing gene ELOVL4 encodes a protein that belongs to a family of proteins functioning in elongation of long chain fatty acids. The purpose of this study is to characterize ELOVL4 protein distribution in mouse, monkey and human eyes. Methods: We raised polyclonal antibody againt ELOVL4 bacterial fusion proteins. The Affinity-purified ELOVL4 antibody was used to perform immunohistochemistry in retinas from mutant and normal ELOVL4 transgenic mice, monkey and human along with the specific outer and inner segment marker antibodies. Results: Elovl4 expression is predominantly enriched in inner segments of photoreceptor from normal mouse, monkey and human. However, ELOVL4 expression in photoreceptors of mutant ELOVL4 transgenic mice had a tendency to follow an aggregated pattern. Conclusions: Our in vivo ELOVL4 expression data are consistent with that of our reported in vitro studies. ELOVL4 transgenic mice will aid our understanding of underlying pathogenetic mechanism of maculae degeneration.

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Chromatin Immunoprecipitation - Microarray Analysis Identifies Transcriptional Targets of the Speech and Language Gene, FOXP2. *E. Spiteri[1], Z. Luo[1], J. Ou[1], B. Ren[2], D.H. Geschwind[1,3].* 1) Department of Neurology, Program in Neurogenetics, UCLA School of Medicine, Los Angeles, CA; 2) Department of Cellular and Molecular Medicine, University of California, San Diego School of Medicine; 3) Department of Psychiatry and Center for Autism Research, UCLA School of Medicine, Los Angeles, CA.

FOXP2 became the first gene identified as the genetic cause for an inherited speech and language disorder when a mutation was identified in individuals of the KE family affected with verbal dyspraxia and language deficiencies. The forkhead binding domain family of proteins, to which FOXP2 belongs, is a group of transcriptional regulators known to be active in development. In the mouse lung, FoxP2 is important for the development of the epithelium and in drosophila FoxP2 is important in the development of the gut. The expression patterns of the FOXP2 mRNA and protein in the brain is becoming well defined. FOXP2 expression starting in post-mitotic neurons is consistent with a role in the development and maintenance of mature neuronal phenotypes. However, the functional role of FOXP2, especially the nature of its transcriptional targets, remains unclear. To better understand the functional role of FOXP2 in brain development, we performed chromatin immunoprecipitation followed by hybridization to a promoter rich microarray (ChIP-chip). To ensure specificity, we made a new polyclonal FOXP2 antibody based on a region with high sequence divergence between FOXP2 and FOXP1. Three replicate experiments were performed in SY5Y cells following peak FOXP2 induction. The targets were confirmed using RT-PCR in SY5Y cells before and after over expression of FOXP2, demonstrating transcriptional regulation by induction of FOXP2. These targets include genes from varying gene ontology categories such as transcriptional activators involved in organogenesis, signal transduction and cell adhesion. Through their relationship with FOXP2 these genes themselves become candidates for evolutionary selection, autism and other speech and language disorders as well as important genes in the development of the brain.

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Humanized xenobiotic response in trans-chromosomic mice containing the human CYP3A cluster. *Y. Kazuki[1], T. Oshima[2], Y. Kai[1], S. Abe[1], M. Takiguchi[1], H. Yamada[1], K. Tomizuka[2], M. Oshimura[1].* 1) Departmentof Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, Tottori University, 86 Nishimachi, Yonago, Tottori 683-8503, Japan; 2) Pharmaceutical Research Laboratory KIRIN Brewery CO. Ltd., 3 Miyabara-cho, Takasaki, Gunma 370-1295, Japan.

Human CYP3A4 is the most abundant P450 isozyme present in human liver and small intestine and participates in the oxidative metabolism of approximately half of all commercially-available drugs, and the CYP gene has clear species-specific inducibility. In order to investigate the role of cis-elements of the human CYP3A cluster and to generate humanized Tc mice that are responsive to a human specific CYP3A inducer, a human artificial chromosome (HAC) containing the CYP3A cluster (CYP3A-HAC) was constructed using the Cre/loxP-mediated chromosome-cloning system. The CYP3A-HAC was then introduced into mouse embryonic stem (ES) cells via Chinese hamster ovary (CHO) cells using microcell-mediated chromosome transfer (MMCT), and healthy chimeric mice containing the CYP3A-HAC were generated from the ES cells. CYP3A4, CYP3A5, and CYP3A7 on the CYP3A-HAC were expressed only in Tc chimeric liver and small intestine, consistent with the expression profile in humans. To investigate whether expression of the human CYP3A genes can be induced in mouse genetic background through trans-regulatory factors, Tc chimeric mice containing the CYP3A-HAC were treated with the human-specific inducer, rifampicin. The CYP3A cluster, particularly CYP3A4 gene expression in the liver of Tc mice with the CYP3A-HAC, was induced by rifampicin. The present data suggest that the endogenous mouse trans-acting factors trans-activated the human CYP3A gene in the Tc mice without human nuclear receptor, hPXR/SXR. In conclusion, the Tc chimeric mice containing the full CYP3A cluster are the first model mice showing interspecific expression differences by introducing cis-acting element rather than trans-reguratory factor. These mice may provide a useful model in which to test novel drugs that induce the CYP3A genes or to identify a novel cis-element in CYP3A4 activation.

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Changes in Molecular Conformation of Expanded Ataxin-3: Experimental Evidence from Small Angle X-ray Scatering (SAXs) Technique. *M.C.C. Garcia[1], C.L.P. Oliveira[2], I. Torriani[2], C.E. Benedetti[3], I. Lopes-Cendes[1].* 1) Medical Genetics, Fac. Ciencias Med / UNICAMP, Campinas, SP, Brazil; 2) Physics Institute GLEB WATAGHIN, UNICAMP, Campinas, SP, Brazil; 3) Synchrotron Light National Laboratory, CAMPINAS, SP, Brazil.

Several dominant inherited human neurodegenerative diseases are caused by expansion of a polyglutamine (polyQ) domain within the respective proteins. This confers a putative dominant toxicity leading to dysfunction and neuronal loss. Expanded polyQ proteins form aggregates within neurons but precisely how it causes neuronal dysfunction and cell death remains unclear. Machado-Joseph disease (*MJD*) is one of the most frequent forms of a neurodegenerative disorder caused by this type of molecular defect in the protein ataxin-3, which physiological and biochemical functions are unknown. Since the function of a protein is closely related to its structure, information on its conformation is very useful for function prediction. Small-Angle X-ray scattering (SAXS) technique is a valuable tool for studying protein conformation in solution, at conditions close to the native state. Recombinant human ataxin-3 with normal (18Q) and expanded repeats (71Q) were expressed in *E. coli* and purified by affinity chromatography. SAXS measurements were performed in samples containing at least 1mg/mL of protein and revealed a value for the molecular weight close to the expected for both isoforms, increasing the reliability of the data obtained. Our results indicated an elongated conformation and the presence of loose parts in the structure. Ab initio calculations allowed the construction of low-resolution three-dimensional (3D) models. Comparison of both isoforms showed that the expanded polyQ tract significantly changes the 3D conformation of the protein and this could be related to the pathogenic effect. Our results showed, for the first time, conclusive evidence of conformational changes related to the pathogenicity of expanded polyQ tracts in human proteins.

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The aggregative properties of proteins with polyalanine domains. *A. Klein, C. Alexander, M. Ebihara, M.J. Dicaire, B. Brais.* Neurogenetique, CHUM - Hopital Notre-Dame, Montreal, QC, Canada.

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disease caused by an expansion of triplet (GCN) encoding a polyalanine domain in the poly(A) Binding Protein Nuclear 1 (PABPN1). PABPN1 is a polyadenylation factor which allows the elongation of the poly(A) tail of each mRNA. The main characteristic of the disease is the formation of intranuclear inclusions in the skeletal muscle fibers. To date, eight other diseases caused by mutations in a polyalanine-coding sequences have been described. Recently several studies showed that seven of the eight proteins involved in the polyalanine diseases form nuclear or cytoplasmic inclusions when overexpressed in cellular models. These results suggest that the polyalanine proteins share a tendency to aggregate. To establish if this is a general property of proteins with polyalanine domains, we chose to study some that have not been implicated in diseases. We selected six transcription factors with variable size (7 to 17 alanines) and position of the polyalanine domain in the protein: TBX2, ID4, BHLHB3, TLE3, PUM2 and ZNF358. We also selected PHOX2B which possess the larger polyalanine domain in human (20 alanines). Immunofluorescence studies allowed us to confirm the localization of the exogenous protein and the eventual inclusions formation in the transfected cells. Our results showed that five of the seven proteins tested form nuclear inclusions when overexpressed, while one formed cytoplasmic inclusions. Size and position of the polyalanine domain do not seem to influence greatly the general aggregation properties. Co-expression studies with PABPN1 showed that even if the proteins form inclusions, they do not co-aggregate. These results confirm the aggregative properties of proteins with polyalanine domains while suggesting that specific sites of nuclear aggregation do not exist.

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Ocular expression profiling and co-localization of Optineurin with its interacting proteins of RAB8, Myosin VI, and FOS in Rhesus Monkey. *T. Rezaie, M. Sarfarazi.* Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT.

Previously, we reported that mutations in the Optineurin (*OPTN*) gene are involved in the etiology of adult-onset primary open angle glaucoma (Science 2002; 295:1077). Recently, we determined expression pattern of this protein in various ocular tissues of human and mouse (Genomics 2005; 85: 131-138). In this study, we aimed to study the endogenous OPTN and its known interacting proteins of RAB8, Myosin 6 (MYO6), and FOS in ocular tissues of Rhesus monkey. Direct interaction of OPTN with these proteins has previously been documented in the yeast two-hybrid system. For OPTN, an anti-peptide antibody previously raised in chicken was used but specific antibodies to OPTN-interacting proteins of RAB8, MYO6 and FOS were obtained commercially. Immunohistochemistry analyses revealed a high expression of OPTN in anterior segment, trabecular meshwork, non-pigmented ciliary epithelium, ciliary muscle, iris constrictor muscle, endothelial cells of blood vessels and Schlemm's canals. Additionally, prominent immunolabeling was observed in retinal ganglion cells, inner and outer plexiform and in the photoreceptor layers. Interestingly, a very high degree of co-localization was observed in ocular tissues of Rhesus monkey between OPTN and its interacting proteins of RAB8, MYO6, and FOS, particularly in the retinal ganglion cells and photoreceptors rods and cones. In silico analyses revealed that for Rhesus monkey, *RAB8b* encodes for 207-aa, *MYO6* for 1285-aa, and *FOS* for 380-aa polypeptide that are, 100%, 98% and 97% identical to their human counterparts, respectively. In summary our study, in addition to OPTN, elucidates specific ocular expression of RAB8, Myosin 6, and the FOS proteins in various ocular tissues of Rhesus monkey. Biological processes in which normal and abnormal copies of OPTN interact with RAB8, MYO6 and FOS still remain to be determined. Further studies also needed to determine if mutations in any of these OPTN-interacting proteins would have a direct involvement in glaucoma optic neuropathy. Supported By: EY-009947 and EY-014959.

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Usher syndrome, audiological and vestibular finding.14 Mexican patients. *F. Castillo-Lorca[1], C.F. Martinez-Cruz[2,3], R. Baez-Reyes[4], L. Gomez-Hernandez[5], A. Garcia-Huerta[1], S.G. Juarez-Garcia[6], G. Garcia-Sanchez[1].* 1) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Instituto Nacional de Rehabilitación; 2) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 3) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 4) Departamento de Genetica. Instituto Nacional de Perinatologia, Mexico, D.F; 5) Servicio de Audiologia.Instituto de la Comunicación Humana/Instituto Nacional de Rehabilitación. E-mail gsanchezg@yahoo.com.mx; 6) Servicio de Neuropsicologia Infantil.Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación.

Usher syndrome is the most common condition that involves both hearing and vision problems. Although the syndrome was first described by Albrecht Von Graefe in 1858, it was named for Charles Usher, a British eye doctor, who believed that the condition was inherited or passed from parents to their children. The major symptoms of Usher syndrome are hearing impairment and retinitis pigmentosa. Some people with Usher syndrome also have balance problems. There are three general types of Usher syndrome. The three types of Usher syndrome are Usher syndrome type 1 (USH1), Usher syndrome type 2 (USH2), and Usher syndrome type 3 (USH3). USH1 and USH2 are the most common types. Together, they account for approximately 90-95 percent of all cases of children who have Usher syndrome..Approximately 3-6 percent of all deaf children and perhaps another 3-6 percent of hard-of-hearing children have Usher syndrome. In developed countries such as the United States, about 4 babies in every 100,000 births have Usher syndrome.In this report we studied the type/ grade of hearing impairment, vestibular function and clinic type of Usher, so we could classify them. Material and methods: We evaluated 14 patients with Usher syndrome. They underwent tonal audiometry, caloric tests and posturography by conventional techniques. Results: We studied 14 patients with different types of Usher syndrome: 8 males (57.1%) and 6 females (42.9%). The youngest was 16 years old and the oldest: 47, with a media of 29 years old; 9 patients (64.3%) had USH1, 4 (28.2%) USH2 and l patient (7.1%) USH3. All patients with USH1 showed profound sensorineural hearing impairment with abnormal caloric and posturographic tests. Patients with USH2 had severe sensorineural hearing loss with normal vestibular tests, and patient with USH3 showed severe progressive deafness with normal vestibular tests. Conclusions: The clinical findings of Usher syndrome are enough to classify each type. Patients with Usher syndrome do not come for an audiologic evaluation at first instance that is why our approach was difficult. In spite of this is a small sample, USH1 was the most frequent type seen.

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Oculocutaneus albinism and congenital sensorineural hearing loss. Mother and son in a Mexican family. *L. Hernandez-Gomez[1], D.O. Gomez-Torres[2], C.F. Martinez-Cruz[3,4], R. Baez-Reyes[5], S.G. Juarez-Garcia[6], G. Garcia-Sanchez[7].* 1) Servicio de Audiologia. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación; 2) Departamento de Investigacion.Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación; 3) Servicio de Comunicación Humana, Departamento de Seguimiento Pediátrico, Instituto Nacional de Perinatologia, Mexico, D.F; 4) Servicio de Pediatría, Hospital General de Zona no. 53 IMSS Los Reyes la Paz. Estado de México; 5) Departamento de Investigacion.Instituto Nacional de Perinatologia, Mexico, D.F; 6) Servicio de Neuropsicologia Infantil.Instituto de la Comunicación Humana/instituto Nacional de Rehabilitacion; 7) Servicio de Genética, Departamento de Cirugía y Medicina Interna. Instituto de la Comunicación Humana/Centro Nacional de Rehabilitación.E mail gsanchezg03@yahoo.com.mx.

Syndrome characterized by total oculocutaneous albinism and congenital severe hearing loss was found in tour children in two sibships in a kindred and described by Ziprkowski and Adam, in 1964. The characteristics of ibis syndrome include (1) autosomal recessive inheritance: (2) albinism of the entire body, including, optic fundí and irides: (3) deficient media eyebrows; and (4) congenital severe sensorineural hearing loss. However, Smith (3) has examined the kindred once again and attests to the validity of a condition now termed oculocutaneous albinism and congenital sensorineural hearing loss, autosomal dominant. We report mother and son with oculocutaneus albinism and sensorineural hearing loss. CASE 1: Male child 4-year-old, is the third child, of young, non consanguineous parents. Hearing loss onset at 3 months of age. Oculocutaneous albinism is observed since birth. ABR performed at 2 years old reported bilateral response at 90dB. Audiometric test showed sensorineural bilateral severe hearing loss. CASE 2: female patient 29-year-old, probandos mother, hearing loss onset at 4-year-old. Oculocutaneus albinism. Audiometric test showed sensorineural bilateral severe hearing loss. Vestibular test were normal.

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***GJB2/GJB6*testing for infant hearing loss from a diverse US population.** *L. Schimmenti[1], A. Martinez[2], M. Teletar[2], N. Shapiro[2], M. Fox[2], B. Crandall[2], Y. Sininger[2], C-H. Lai[2], W.W. Grody[2], C.G. Palmer[2].* 1) Dept Peds/Inst Human Gen, Univ Minnesota, Minneapolis, MN; 2) Dept of Psychiatry and Biobehavioral Sci,Lab Medicine and Pathology, Pediatrics, Otolaryngology, Human Genetics, UCLA, Los Angeles, CA.

Mutations in the genes *GJB2/GJB6* are associated with up to half of the cases of non-syndromic hearing loss in some populations. We are in the third year of a longitudinal study in a diverse California population to determine the feasibility and impact of introducing testing into newborn hearing screening protocols with the hypothesis that genetic testing will enhance diagnostic efforts and intervention. To date, 74 infants and toddlers with non-syndromic hearing loss are enrolled in the study. For all infants, buccal cell derived DNA is used to sequence both exons of *GJB2* and to assay for del(*GJB6*-D13S1830). Using these methods, hearing loss can be explained in 19 infants. (26%) by the presence of biallelic mutations in *GJB2/GJB6*. The three most common genotypes are 35delG/35delG (8), 35delG/167delT(3) and V37I/V37I (3). We also identified 5 infants with various biallelic *GJB2* hearing loss associated variants (35delG/311del14, 235delC/235delC, 35delG/K168X) including two with double heterozygosity for del(GJB6-D13S1830)(167delT/delGJB6, 35delG/delGJB6). In the remaining 55 infants, 4 infants are identified with only one hearing loss associated allele (35delG/wt(2), Y152X/wt(1) and S139N/wt(1)), 15 with known polymorphisms (V27I/wt (9), V27I/V27I(2), V153I/wt(1), M34T/wt(2), V27I,E114G/wt(1)), and 36 with only wildtype alleles. When the bialleic data was broken down by ethnic group (where both parents are of the same ethnic group), mutations are identified in 10/24 (41%) of non-Hispanic Caucasian infants, 4/9 (44%) of Asian infants and 2/29(7%) of Hispanic infants. In infants of mixed heritage, (non-Hispanic Caucasian/Hispanic), we find 3/9 with bialleic mutations in *GJB2*. We show that mutation testing in infants from a diverse Los Angeles population is feasible and robust. However, the low rate of *GJB2/GJB6* mutations warrants further study to identify causes of hearing loss in infants of Hispanic heritage.

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The frequency of the *GJB2* V27I polymorphism in the Los Angeles Mexican American population. *M. McCarra[1], A. Martinez[2], M. Teletar[2], N. Shapiro[2], M. Fox[2], B. Crandall[2], Y. Sininger[2], W.W. Grody[2], C.G. Palmer[2], L.A. Schimmenti[1].* 1) Pediatrics, Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 2) Psychiatry and Biobehavioral Sci, Lab Med Pathology, Pediatrics, Human Genetics, Otolaryngology, UCLA, Los Angeles, CA.

Recessive mutations in *GJB2* are the most commonly identified genetic cause of non-syndromic hearing loss and are associated with up to half of all autosomal recessive non-syndromic hearing loss in Caucasian populations. In a longitudinal prospective study of infants in Los Angeles with apparent non-syndromic hearing loss, it was noted that the V27I (79G>A) variant was commonly identified in a number of Mexican-American infants but rarely observed in non-Hispanic Caucasian infants. Of 18 infants with sensorineural hearing loss who had both parents of Mexican heritage, the V27I polymorphism occurred as the only sequence variant in 8 of 36 chromosomes (22%); four being heterozygous and two being homozygous. As a control for this group of infants and to find the population frequency of V27I, one hundred DNA samples from Mexican American individuals from Los Angeles were obtained from the Coriell Institute. In the control group, at least 3 grandparents were born in Mexico. Ninety-nine samples were amplified with *GJB2* specific primers, and assayed by restriction digest for the presence of the 79G>A. We determined that the V27I variant occurred in 46 of 198 chromosomes (23.2%); 36 being heterozygotes and five being homozygotes, confirming that V27I is a polymorphic variant with very high frequency (0.23) in the Mexican American control population and is not a hearing loss-causing allele.

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Correlation of acute GVHD with HLA sequence and structural dissimilarity after unrelated hematopoietic stem cell transplantation. *S. Hwang[1], Y.S. Heo[2], J.J. Seo[3], J.W. Lee[4], H.B. Oh[1].* 1) Laboratory Medicine; 2) CrystalGenomics, Inc; 3) Pediatrics; 4) Internal Medicine, Univ. of Ulsan, College of Medicine, Asan Medical Ctr, Seoul, Korea.

High-resolution HLA mismatch is known to be major cause of acute graft-versus-host disease (aGVHD) after hematopoietic stem cell transplantation (HSCT). Direct allorecognition of T cell is the major mechanism for aGVHD. Hypothesis of this study was that the severity of aGVHD would increases according to structural difference between HLA alleles. To estimate the difference between a donor/recipient pair, we used two dissimilarity score matrixes of amino acids. One was Risler's distance matrix (Bone Marrow Transplant 2004:165-9) which is based on evolution. The other was invented to access structural dissimilarity in aspect of space-electrostatic bonding and hydrogen bonding. Mismatches occurred in the region of peptide-binding and TCR-contact were given more penalties (score). We developed a web-based system to get dissimilarity score (www.koreanhla.net). The system was designed to be tailored by users, which meant that nearly all parameters could be changed easily on the web with administrator's permission. Correlation between the dissimilarity score and the grade of aGVHD after HSCT was analyzed in thirty-eight donor/recipient pairs. The sequence and structural dissimilarity scores of HLA-A, -B and - DR loci correlated significantly with the grade of aGVHD ($r = 0.460$, $r = 0.356$, respectively; $p = 0.004$, $p = 0.031$, respectively). It was also found that substitution at residue 99 of HLA -A significantly related to the prevalence of aGVHD. It could be suggested that the dissimilarity scores which reflect HLA difference in aspect of sequence or structure correlate well with the severity of aGVHD. Larger sample size is necessary to confirm our result. Web-based system will give researches chance to use our system, by which our system will be further refined.

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Genetics Home Reference: translating genetic data into educational materials. *J.A. Mitchell[1], C. Fomous[2], J. Fun[2], S. Morrison[2].* 1) Med Informatics, Univ Utah Sch Medicine, Salt Lake City, UT; 2) Nat Library Medicine, NIH, Bethesda, MD.

The Genetics Home Reference (GHR) website (**http://ghr.nlm.nih.gov**) fills a unique niche by using plain language to interpret the health implications of the Human Genome Project. Prior to GHRs launch in April 2003, online genetic resources focused on the needs of genetics professionals. Consumers seeking information about inherited disorders, however, reported that genetics websites were often confusing, difficult to navigate, and hard to understand. GHR was developed to address the growing need for online genetics information in lay language. GHR uses innovative strategies developed through informatics research to manage the large and expanding body of genetics information. Automated techniques integrate information across data sources, assist data interpretation, and increase the accuracy of search results. GHRs architecture collects information as structured data with embedded relationships and presents this data in a simple, document-based format. Fields in the structured data can be mapped to other databases, controlled vocabularies, and ontologies. For example, condition names are mapped to Medical Subject Headings (MeSH) to improve the accuracy of search results. Mining databases, such as Entrez Gene, OMIM, and Gene Ontology streamlines content development by automatically extracting information for content developers to use. The marriage of bioinformatics data and consumer-oriented content has been fruitful. Content on the GHR site doubled over the past year; currently, information is available for more than 200 conditions and 250 genes. Site traffic has increased fourfold over the past 2 years. Tracking data show that all features of the site are used. Initial evaluation found very high overall user satisfaction. GHR is a successful example of using informatics tools to produce an educational resource that meets consumers needs. Continued growth of the site will guide the application of informatics techniques and drive new research to assist content development, ensure accuracy, and help consumers navigate the complex world of genetics.

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Fetal Aqueductal Stenosis: Prenatal diagnosis and postnatal outcome/autopsy findings. *E. Ying[1], M. Sgro[1], S. Keating[3], D. Chitayat[2].* 1) Pediatrics, St. Michael's Hospital, Toronto, Ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology; 3) Department of Pathology, Mount Sinai Hospital. University of Toronto, Toronto,Ontario, Canada.

Background: Aqueductal stenosis is a rare finding. Prenatal diagnosis of aqueductal stenosis is defined by the ultrasound finding of enlargement of both lateral and third ventricles with a normal sized 4th ventricle. Objectives: To report and describe our experience of the etiology and outcomes of fetuses prenatally diagnosed with aqueductal stenosis on ultrasound. Methods: Over a 15 year period (1992-2005), all pregnancies with the finding of aqueductal stenosis seen at a tertiary regional prenatal diagnosis centre were obtained through a database. The outcomes including laboratory (serologies, platelet antibodies, L1CAM gene mutation, and chromosome analysis), delivery records, autopsies and neonatal follow up were gathered by chart review. Results: 29 patients with fetal ultrasound findings of aqueductal stenosis were seen at the Prenatal Diagnosis Program. The mean gestational age at diagnosis was 20.2 weeks (range 15-39.7 weeks). Follow-up was available in 26 cases. Four (14%) had chromosomal abnormalities (2- trisomy 21; 1- trisomy 18 and 1- 45,X). In 4 cases (14%) L1CAM gene mutation was detected. Multiple anomalies, including renal and cardiac, were found in 10 cases including one with Walker-Warburg syndrome and one with 3C syndrome. 5 had additional central nervous system abnormalities including cerebral dysgenesis, myelomeningocele and lissencephaly. 23 pregnancies were terminated. One child who is alive (45, X) is severely delayed. Conclusions: Our report highlights the etiological hetegeneity of aqueductal stenosis detected prenatally and the need for a thorough autopsy, chromosome analysis and mutation analysis of the L1CAM gene in males and 45,X females to try and delineate the diagnosis and thus provide accurate genetic counseling.

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Still lumping after 14 years: OFD-VI, Hydrolethalus and Pallister-Hall syndromes. A diagnostic dilemma for recurrence risk counseling. *D.M. McDonald-McGinn[1], N. Unanue[1], S. Saitta[1], S. Halbach[1], S. Purandare[1], M. Muenke[2], H. Honkala[3], L. Biesecker[2], W.B. Dobyns[4], E.H. Zackai[1].* 1) Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine; 2) NIH; 3) NPHI of Helsinki; 4) U of Chicago.

We previously reported a fetus with overlapping features of OFD-VI, Hydrolethalus (HYL) and Pallister-Hall (PH) syndromes (Muenke 1991). Fourteen years later we describe another such patient with significant overlap including: ocular colobomas and an occipital keyhole defect as seen in HYL, a molar tooth and multiple frenula as described in OFD-VI, a hypothalamic hamartoma consistent with OFD-VI and PH, and cerebellar vermis hypoplasia, epiglottal abnormalities and lower extremity pre-axial polydactyly as reported in all three. In 1991, there was no definitive way to distinguish the above syndromes. Because of this insolvable overlap Verloes suggested a phenotypic classification called the multiplex syndrome. This impacted recurrence risk counseling though, as PH is dominant while HYL is recessive. Progress has been made, however, as mutations have now been identified in PH (GLI3-Johnston 2005) and HYL (HYLS1-Mee 2005) and these studies are pending in our patient. Since many of our probands features overlapped with OFD-VI (presumed recessive) we reviewed the brain findings in 3 of our patients with OFD-VI (Patients 1 and 2 - Muenke 1990) and their long-term outcome. All had a molar tooth, hypothalamic hamartoma and vermis hypoplasia. The prognosis correlated with the severity of the brain findings: the 18 year old is non-verbal/non-ambulatory, the 28 year old is normal intellectually and the 4 year old has significant delays. Based on our probands brain and airway findings, the long-term prognosis, regardless of the classification is considered poor. However, the outcome of mutational analysis may offer definitive recurrence risk counseling. Thus, this report emphasizes the importance of CNS abnormalities in this spectrum of disorders as a prognostic indicator, whereas the specific mutations will ultimately determine the recurrence risk.

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Evaluation of unclassified DNA sequence variants of the BRCA1 and BRCA2 genes. *R.K. Schmutzler[1], B. Wappenschmidt[1], M. Brosig[2], A. Meindl[3], German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC).* 1) Dept. Ob/Gyn, University of Cologne, Germany; 2) Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany; 3) Dept. Ob/Gyn, Technical University of Munich, Germany.

Purpose We aim to categorize unclassified DNA variants (UCVs) i.e. missense mutations of the BRCA1 and BRCA2 genes according to a multifactorial likelyhood-ratio (LR) model (Goldgar et al., AJHG 75:535-544, 2004). **Material and Methods** Within the GCHBOC 91 unique BRCA1 and 143 unique BRCA2 UCVs have been detected in 3081 independend index cases. Provided that a UCV does not exert an intermediate risk we perform co-occurrence, co-segregation, biochemical, interspecies variability and loss of heterozygosity (LOH) analysis. Calculation of LOH LRs is performed under the assumption that 95% of hereditary breast cancers but only 20% of sporadic breast cancers reveal LOH of the BRCA chromosomal regions. **Results** We first evaluated the model by analysing the BRCA2 UCV S384F by co-occurrence, co-segregation and LOH analysis and revealed a combined LR of $1.4x10^{-8}$ in favour of neutrality. Subsequent analysis of co-occurrence revealed highly significant LRs of <0,001 in favour of neutrality for 5 UCVs in BRCA1 (L6V, R7C, I15L, E23Q, P1099L) and 3 in BRCA2 (N289D, L1019V, V2969M). In additional 5 cases exclusion of LOH of the BRCA wildtype allele in 1 breast carcinoma per family (BRCA1: L668F, R866C; BRCA2: R2108C, R3052W, F3065L) led to an uncertain LR of 0.0625. In 1 family with the BRCA2 UCV I1446T located in the Rad51 binding domain, 2 breast carcinomas revealed maintenance of the wildtype allele leading to a LR of 0.0039 in favour of neutrality. Biochemical and interspecies analysis of these UCVs were of uncertain significance. **Conclusions** The Goldgar model provides a valuable approach for the evaluation of UCVs and so far 6 UCVs could be classified as being neutral. As co-occurence is the single most informative factor large data sets strongly promote clarification of UCVs. The remaining UCVs require additional material from the families for co-segregation and LOH analysis and are currently under investigation.

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MRI fails to detect invasive breast cancer in a BRCA2 positive patient. *J.S. Wilbur, T.R. Sousa, R.D. Legare.*
Cancer Risk Assessment and Prevention Program, Program in Women's Oncology, Women & Infant's Hospital, Providence, RI.

Introduction: Breast magnetic resonance imaging (MRI) is a relatively novel screening method for women with a familial or genetic predisposition to breast cancer. Since 2000, six noteworthy publications have supported the fact that breast MRI is significantly more sensitive in detecting breast cancer in women at hereditary risk. Case Study: A heathly 44-year-old woman was referred to the Cancer Risk Assessment and Prevention Clinic because she was found to have inherited the BRCA2 deleterious mutation, R2520X. Following comprehensive cancer genetic counseling, which included the option of annual breast MRI screening, the patient decided to pursue prophylactic bilateral mastectomy (PBM). Prior to her PBM, a bilateral mammogram and breast MRI were performed, both of which were negative. The mammogram, performed five months prior to surgery, showed scattered fibroglandular densities in both breasts with no significant masses or calcifications. This mammogram was interpreted by a Board Certified Radiologist as well as a digitized computerized mammography reading program. The breast MRI was performed using standard breast protocol following intravenous administration of gadolinium three months prior to surgery. There was no evidence of mass-like or nonmass-like enhancement or lymphadenopathy. There were scattered areas of enhancing fibroglandular parenchyma with no evidence of malignancy and a BIRADS score of one. Despite her negative mammogram and MRI, the proband pursued prophylactic bilateral skin sparing mastectomy with immediate tram flap reconstruction. Post operatively, pathology identified a right breast TIcN1MX stage II ductal carcinoma, having extensive lymph-vascular invasion. The tumor was estrogen receptor (ER) positive, progesterone receptor (PR) positive, and Her-2/Neu negative by FISH. The patient is currently undergoing chemotherapy treatment. Conclusion: Current publications support breast MRI screening to be an extremely valuable tool in high risk popultations. However, while breast MRI sensitivity is considered superior to other screening modalities, it too has limitations and is worthy of further study.

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Empiric recurrence risks for congenital solitary kidney. *E. McPherson.* Department of Medical Genetics, Marshfield Clinic, Marshfield, WI.

Congenital solitary kidney (CSK) affects about 1/1000 persons while bilateral renal agenesis (BRA) affects about 1/5000. Because CSK is significantly increased in the parents of infants with bilateral renal agenesis (BRA) and in the mothers of fetuses with non-lethal renal anomalies on ultrasound, there is reason for concern that the offspring of CSK patients may be at risk for both unilateral and bilateral renal anomalies, but no empiric data exist. The aim of the current study was to gather empiric data concerning the risks of CSK and BRA in close relatives of individuals with CSK.

Through a computerized review of records from 1979 to the present in a large multispecialty clinic, 206 subjects with CSK (defined as presumably congenital absence or severely reduced function of one kidney) were identified and 82 agreed to participate. They completed a questionnaire regarding renal and related urogenital anomalies in relatives. The numbers affected were as follows:

offspring 14/144(10%); parents 5/164(3%); siblings 4/234(2%)

Because subjects are unlikely to be aware of all non-lethal renal anomalies in their relatives, actual risks may be greater. The subjects report no cases of BRA among their relatives. Significant under-diagnosis of lethal renal anomalies is unlikely because the incidence of stillbirth was only slightly increased (1.8%) and the incidence of miscarriage was comparable to the general population (12%).

The minimum empiric risk for CSK and related anomalies in offspring of CSK patients is 10%, which is slightly less than the reported incidence of CSK in parents of fetuses with non-lethal renal anomalies. Lower risks in parents and siblings may represent underascertainment, but this mirrors the situation when family histories are obtained by interview. The incidence of BRA in relatives of CSK probands is apparently low, suggesting that either CSK is usually a separate disorder or the incidence of BRA relative to CSK is too low to be detected in the current study.

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Y Chromosome long arm (Yq) Microdeletions in Idiopathic Male Infertility. *E. Fernández[1], F. Alvarez-Nava[2], L. Borjas[2], J. Osuna[3], W. Zabala[2], M. Zambrano[4], MG. Portillo[2].* 1) Inst de Inv Clínicas, Universidad del Zulia, Maracaibo, Maracaibo/Zulia, Venezuela; 2) Unidad de Genética Médica, Facultad de Medicina Universidad del Zulia (LUZ); 3) Unidad de Endocrinología del Hospital universitario de Mérida; 4) Cátedra de Bioquímica Clínica, Escuela de Bioanálisis, Facultad de Medicina-LUZ.

Today infertility is a major health problem affecting about 10-20% of couples. Male factor is assumed to be responsible in about 50% of the infertile couples. Both qualitative and quantitative abnormalities in sperm production are responsible in 40% of the infertile population. The origin of reduced testicular sperm function is unknown in about 60-70% of cases. Micro-deletions in the Yq are known to represent the pathogenic mechanisms for infertile males. Four differents non-overlapping regions designated as AZFa, AZFb, AZFc and AZFd are located in interval 5-6 of Yq, and are associated with impaired spermatogenesis in humans. To determine the prevalence and distribution of Y chromosomal microdeletions in venezuelan males with idiopathic infertility, chromosomal, Y-microdeletion, seminal and histological analyses were carried out in 30 venezuelan males with idiopathic azoospermia or oligoospermia. Yq Microdeletions were tested by mutiplex PCR, and were detected in 2 patients (6.7%). One patients had Yq microdeletions on AZFc-d and other had almost the entire AZF region with undetectable mosaicism (45,X/46,XY) on a first cytogenetic analysis. Affected patients had azoospermia and the histological findings the SCOS type I in one patient. The frequency and distribution of AZF microdeletions in venezuelan patients were similar to other populations with different ethnical or geographical origin. Patient selection criteria seem to be showed most important factor on the prevalence of microdeletions. Genetic evaluation in azoospermic men is recommended before to realize other diagnostic tests because of a high frequency of cytogenetic and genetic abnormalities among these patients with Yq microdeletions. Carrying patients of microdeleciones Yq can present an increase in the incidence of cellular lines 45, X in his karyotype.

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Pyridoxine responsive seizures: phenotypic and pathogenic variability of febrile seizures, hypophosphatasia and hyperphosphatasia with neurologic defect. *P. Hwang[1], M. Thompson[2].* 1) Neurology, North York General Hospital, Toronto, Ontario, Canada; 2) Dept. Pharmacology, University of Toronto.

We present a series of paediatric cases with pyridoxine responsive seizures of variable aetiology. In the context of the more common pridoxine responsive neonatal seizures (infantile spasms in Japan), and the hypophosphatasia syndromes, we present data collected from families affected by pyridoxine responsive hyperphosphatasia with neurologic deficit or HND (MIM#239300). Considerable clinical heterogeneity within these disorders belies the fact that they are commonly responsive to pyridoxine. The genetic and physiological basis of the pyridoxine responsive hypophosphatasia syndromes contrasts with that proposed to explain hyperphosphatasia with neurologic deficit. In addition, the hyperphosphatasia seen in HND is not accompanied by abnormalities of bone, liver metabolism, or altered skeletal architecture. As in the other disorders, affected siblings and/or consanguinity suggest autosomal recessive inheritance. Unlike the hypophosphatasias and neonatal spasms, the underlying pathogenesis of HND is unknown. We describe a series of cases with heterogeneity and review the clinical features in the context of the other pyridoxine responsive diseases. Electroencephalographic (EEG) assessment is used to contrast the pyridoxine responsive syndromes. Whether or not febrile seizures or hyophosphatasia are evident, we suggest that phenotypic delineation of epileptic encephalopathy in infancy, childhood and adolescence should involve assays for: 1) alkaline phosphatase; 2) pyridoxine-EEG challenge. We contrast the genetic disruptions that underlie the metabolic errors in these disorders and suggest strategies for isolating the genes that contribute to the heterogeneity of HND and other pyridoxine responsive disorders.

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Maternal reports of family history from the National Birth Defects Prevention Study, 1997-2001. *R.Fisk Green[1], R. Olney[1], J. Reefhuis[1], L. Botto[1], P. Romitti[2].* 1) NCBDDD, CDC, Atlanta, GA; 2) Dept Epidemiology, Univ Iowa, Iowa City, IA.

Enhancing family history (FH) use in pediatrics, in which parents provide most information, requires exploring what FH data could be obtained. We analyzed FH responses from the National Birth Defects Prevention Study (NBDPS) using data from completed interviews of mothers of children with birth defects (n=9331) and of control children (n=3390) with 1997-01 delivery dates. Mothers were asked if they, the father or other relatives had a birth defect or health problem at birth and, if so, what it was. We could not confirm FHs, so differences in accuracy or FH risk could not be determined. Of respondents with completed interviews, 17% reported an FH of birth defects, 5% genetic disorders, 10% developmental disabilities and 24% other conditions. Reports of birth defects in mothers and fathers were nearly equal in number, and defects were reported more for 1st and 2nd degree relatives than 3rd. Further analyses included only 1st and 2nd degree relatives. Univariate analyses indicated that, as expected, case mothers were nearly twice as likely as control mothers to mention an FH of birth defects (OR=1.9 [95% CI 1.7, 2.2]). White, non-Hispanic maternal race compared with all other races and ethnicities was associated with more reports of an FH of birth defects (OR=1.7 [1.5, 2.0]), as was maternal education of >12 years (OR=1.2 [1.1, 1.4]), English spoken at home (OR=2.8 [2.3, 3.3]) and an annual family income >$20K (OR=1.3 [1.2, 1.5]). Other factors associated with reporting an FH of birth defects included the father contributing to the interview (OR=1.4 [1.1, 1.6]) and the mother reporting factors she thought might cause birth defects (OR=1.6 [1.4, 1.7]). We assigned a level of detail (high, medium, low) to responses indicating a birth defect FH and found that a high level of detail was associated with being a case mother (OR=1.7 [1.1, 2.6]) and speaking English at home (OR=1.5 [1.1, 2.1]) but not with maternal race, education or family income. Our results suggest that demographic factors might affect reporting of FH, which could be considered in developing pediatric FH tools.

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NIPBL Mutational Analysis in a Cornelia de Lange Syndrome Family with Two Affected Siblings and Normal parents. *D.M. Niu[1], N.C. Lee[1], J.Y. Wu[2], K. Kosaki[3].* 1) Pediatrics, Taipei Veteran General Hosp, Yang-Ming University, Taipei, Taiwan; 2) Department of Medical Research, China Medical College Hospital, Taichung; 3) Division of Medical Genetics, Keio University School of Medicine, Japan.

Cornelia de Lange syndrome (CdLS [MIM 122470]), is a clinically heterogeneous developmental disorder characterized by facial dysmorphia, upper-extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities. Mutations in NIPBL have recently been reported to cause CdLS. Mutations in this gene can cause autosomal dominant inheritance. Recently, we found a family in which a boy and a girl in the same sibship show variable manifestations of CdLS. The brother had severe congenital heart disease and expired at age of one. The sister only had a mild patent ductus arteriosus (PDA) with spontaneous closure. Both parents are healthy and phenotypically normal. Mutational analysis of NIPBL gene was performed for the affected sister and both parents. A missense mutation (D2433G) at exon43 was identified at the affected sister, but not in both parents. This study reminds us that germline mosaicism should be considered for CdLS even in the parents who have normal phenotype.

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A Survey of the Genetic Information Needs of Clients and Clinicians at Clinics in Washington, DC. *K. Christensen, E. Alfano, J. Martin, R. Caligiuri, R. van der Riet, N.T. Robinson, L. Wise, S.F. Terry.* Genetic Alliance, Washington, DC.

Purpose: To determine what information individuals from underserved and underrepresented communities need in the course of general medical care, what their health providers consider important and in what form that information is accessible.

Methods: We surveyed the literature, conducted surveys and focus groups of individuals who access Washington DC clinics for services. In addition, we surveyed clinicians who serve these underserved and underrepresented individuals. We qualitatively analyzed this information.

Results: Individuals receiving information in these clinics need consumer friendly, accessible materials. The literacy level of these materials must be appropriate for a wide range of clients. Consumer perspectives of what constituted critical information differed from clinician perspectives. Consumers achieved a high level of conceptual understanding when materials were made accessible.

Conclusions: In addition to understanding the socioeconomic and cultural attributes of clients in health clinics that serve underserved and underrepresented communities, material development for these communities must also integrate complex genetic information in an accessible manner. As a result, we developed a manual for clinicians to share genetic information with their patients.

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Utilization of genetic counseling services by surgical oncologists: education a must. *D.M. Agnese, H. Hampel, C. Eng.* Internal Med/Human Genetics, Ohio State Univ, Columbus, OH.

Genetic counseling services are available at many institutions, however referral patterns differ among medical professionals. Perhaps the most widely used is cancer genetic counseling. The purpose of this study was to determine basic knowledge of and utilization of cancer genetic counseling services by surgeons specializing in oncology. Prior to the annual meeting in March 2004, where an educational session on cancer genetics was planned, a questionnaire was sent to the membership of the Society of Surgical Oncology. This survey evaluated the type and volume of practice, and basic information about knowledge of genetic consultation availability and genetic testing. The survey was electronically sent to 1512 members of the Society of Surgical Oncology. It is unknown how many were unopened or not received. There were 364 responses. The majority (71.3%) were academic surgeons. The majority saw more than 50 new cancer cases annually, and 31.8% saw more than 200 new cancer cases annually. Nearly all (98.1%) collected family history on these patients. Genetic consultation services were available in the majority of centers (82.6%). Despite this availability, only 56.1% referred frequently, while 38.3% referred occasionally and 5.6% rarely referred. In addition, 60% of those surveyed had ordered genetic testing on their patients. Members of the Society of Surgical Oncology, a predominantly academic group of surgeons with a particular interest in cancer surgery, do collect family history information when evaluating new cancer patients, although the extent of this history and its accuracy are not known. Surprisingly, although genetic consultation services are available in the majority of institutions, many surgeons have ordered genetic testing on their own patients. The non-respondents may represent a group even less likely to refer, and as such, our estimate of the frequency of counseling usage may be even lower. Further education is necessary in this group to reinforce the importance of genetic counseling prior to ordering testing.

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The use of Concept Mapping to assess genetic counseling needs of prenatal patients. *A. Cronister[1], S. Bhatt[1], D. Cutillo[1], T. Cousineau[2].* 1) Genzyme Genetics, Phoenix, AZ; 2) Inflexxion, Inc, Newton, MA.

Purpose: Integral to the successful delivery of prenatal genetic services is providing patients with information they deem relevant. The study was designed to assess what patients perceive as important information in a routine prenatal genetic counseling session and compare this to what health experts consider most relevant. **Methods**: A structured program planning procedure called Concept Mapping (CM), was used to systematically analyze qualitative information generated from patients who had received genetic counseling (n=11), Genzyme genetic counselors (n=12) and perinatologists (n=8). This technique allowed for a direct and quantifiable comparison of differences among these various groups. **Results**: 61 statements were generated and sorted into 7 distinct cluster maps: Prenatal diagnostic testing, Prenatal screening, Genetic counseling information, Personalized genetic risk assessment, Carrier and ethnicity screening, General genetics information, Follow-up information and resources. A pattern match was generated using The Concept System software (Concept Systems, Inc, Ithaca, NY), allowing a comparison of how groups rated the content statements. Results demonstrated a high level of agreement between genetic counselors and physicians (r=.81). Moderate though strong agreement was noted between genetic counselors and patients (r=.58), which was greater than agreement between physicians and patients (r=.48). While all three groups rated information on testing and procedures as highly important, patients gave high importance ratings to most items overall and to wanting specific contact information from Genzyme, suggesting a consumer need for easy access to information. Data regarding all aspects of the CM process will be presented. **Conclusion**: CM allowed for direct and quantifiable comparison between patients and providers. Overall, there was high correlation between groups suggesting that patients, genetic counselors and physicians agree on the most important aspects of prenatal genetic counseling. Concept Mapping is a useful tool in the endeavor to discover efficient and effective ways to both educate patients and provide personalized risk assessment.

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Psychosocial health service implications for genetic testing: A clinical and training needs assessment. *M. Cappelli[1], M.J. Esplen[2], J. Bottorff[3], B. Wilson[4], J. Allanson[1], J. Carroll[5], M. Dorval[7], C. Shuman[5], J. Millar[9], M. Bell[6], P.J. McGrath[8], M. Mullen[1], E. Humphreys[1], E. Wouterloot[1].* 1) Children's Hospital of Eastern Ontario & University of Ottawa, Ottawa, ON; 2) Toronto General Research Institute & University of Toronto, Toronto, ON; 3) University of British Columbia, Vancouver, BC; 4) University of Ottawa, Ottawa, ON; 5) University of Toronto, Toronto, ON; 6) Credit Valley Hospital, Mississauga, ON; 7) University of Laval,Laval, QC; 8) Dalhousie University, Halifax, NS; 9) Nova Scotia Dept of Health, Halifax, NS.

There is wide consensus that implementation of any new genetic services should be accompanied by a complementary psychosocial framework to ensure that patients understand as fully as possible the information they are being given and to enhance psychological adjustment to the implications of such information. Despite the acknowledged importance, an environmental scan and review of the literature identified no reports specifically addressing this issue. We conducted a Canadian national clinical and training needs assessment to determine the level of psychosocial need, as perceived by clinicians in direct contact with patients, identify the types and level of psychosocial services currently available, determine if these sufficiently meet current and projected future needs, and identify options for meeting needs. A survey was conducted(mail-out questionnaires and semi-structured interviews) of all Canadian geneticists (n=47) and genetic counselors (n=78) (total clinician response rate 64%;), medical directors of regional genetic centres and their satellite sites (16/21; 76%), genetic residency programs chairs (4/6), fellowship chairs (3/6), and directors of university genetic counseling training programs (3/3). Across all groups, the findings showed that there is a need for additional training in assessing and screening for psychosocial distress, more knowledge on how and when to triage for more specialized mental health care, and also, additional training for front-line staff on initial psychosocial interventions. A follow-up national study is now in progress addressing these issues.

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Family History Tools for Underserved and Underrepresented Communities. *N.T. Robinson[1], S.F. Terry[1], P. Kyler[2], M. Lloyd-Puryear[2], Family Health History Coalition.* 1) Genetic Alliance, Washington, DC; 2) Genetic Service Branch, Maternal and Child Health Bureau, HRSA, HHS.

Purpose: To develop a toolkit to make family health history tools accessible to underserved & underrepresented communities (UUCs).

Methods: We convened a Family Health History Coalition - composed of major organizations working on family history tools. These included: the Genetics Services Branch of the Maternal & Child Health Bureau, Office of the Surgeon General, Centers for Disease Control and Prevention, National Institutes of Health, American Society of Human Genetics, American Folklife Center, Institute for Cultural Partnerships, National Society of Genetic Counselors, Genetic Alliance, UUC nonprofits, biotech companies & others. These organizations analyzed existing family history tools; conducted research, including focus groups, to create a guide to match UUCs with the best tool for them and refine that tool to meet their needs; & developed a plan to disseminate this information to UUCs and the field.

Results: A comprehensive survey of UUC needs in taking family health history; a thorough analysis of the strengths & weaknesses of tools in meeting those needs; & the outline of a resource to aid UUCs in taking better family health histories.

Conclusions: UUCs have unique needs in taking a family health history. These tools should be adapted to allow greater access, thereby reducing health disparities.

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What do patients want: Informed consent models for genetic carrier testing. *K. Ormond, M. Iris, S. Banuvar, J. Minogue, S. Elias.* Northwestern Univ, Chicago, IL.

Recently, there has been an increase in the number of conditions for which patients can undergo genetic carrier testing. This raises the question of how best to provide patients with pre-test informed consent. Clinical approaches vary from a minimalist model (where patients are told various tests exist, with details provided if they test positive) to a model where patients are given data about all conditions to aid in decision-making. Little data exists as to patient preferences, or the impact on decision-making. This study assessed the knowledge and preferences of pregnant patients and male partners surrounding informed consent for carrier testing, and reactions towards two models of informed consent. Seven high-literacy focus groups were conducted to date, including 24 women and 13 men, from Caucasian, Latino and African American Backgrounds. Groups were moderated by non-genetics social scientist, transcribed and analyzed using ATLAS. Many groups agreed that carrier testing is perceived as relevant to the babys health, and minimized the focus on ones own genetic material. Overall, most groups felt that some balance between details and brevity was optimal, recognizing that anxiety can occur when patients are provided with too much information and that the wide range of tests offered during pregnancy often led to confusion. Women also felt that it was useful to have time to discuss carrier testing with their partners. Carrier testing was seen as different from other routine blood tests conducted in pregnancy. Critical areas for the informed consent process included (1) details about the conditions and risk of being a carrier (2) logistics of testing, (3) next steps if test positive, and (4) prognosis, options and resources if child was affected with disease. A list of reasons why people decide to have or not have testing was also proposed. While more data needs to be collected from low literacy groups, it will be useful to develop model consent programs and prospectively assess their impact on informed consent and patient satisfaction, both when positive and negative results are received. Funded by Friends of Prentice.

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Attitudes on fragile X mutation carrier testing from women identified in a general population survey. *A. Anido[1], L.M. Carson[2], S.L. Sherman[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Emory Transplant Cetner, Emory Univ, Atlanta, GA.

Fragile X syndrome (FXS) is primarily due to a CGG repeat expansion found in the FMR1 X-linked gene. In a previous study, we conducted focus groups with women to assess their attitudes towards FXS carrier screening. In this follow-up study, we conducted in-depth interviews of general population reproductive-age women who were identified as carriers. We explored their attitudes toward testing for carrier status of the fragile X mutation. These women underwent screening primarily to participate in a research project rather than in search of a diagnosis. As such, these women were wholly unprepared for positive carrier results. Their responses about their results and carrier screening, in many cases, were being worked out over the course of the interview itself. The most salient finding of this work is the apparent lack of relevance of carrier status to these women. Many expressed that although the information could be relevant in the future, it is not relevant at this stage of their lives in terms of family planning (either with respect to having unaffected offspring or to premature ovarian failure) and personal relationships. Although issues of abortion seemed prominent in the focus groups, we found that carrier status did not have an apparent effect on womens attitudes about termination. We hypothesize this may be related to the fact that women had not processed their new carrier status and had not related it to previously-formed personal opinions. The findings of this work have significant implications for genetic counseling and population screening. Genetic counselors should be mindful that general population women may not recognize the immediate importance of their carrier status even when literature is discussed prior to providing a sample. In addition to standard counseling, counselors should identify the reproductive life stage of the women receiving the new information, help identify when this information would be useful, and personalize a flow sheet of specific services that will be more relevant to the women as their carrier status becomes more relevant.

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How are Minnesota parents informed about newborn screening? *K.J. Johnson[1], S. Berry[2], D. Bartels[2], B. Leroy[2], L.A. Schimmenti[2].* 1) Genetics, Mayo Clinic, Rochester, MN; 2) Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Newborn screening (NBS), performed in the United States since the early 1960s,remains one of the largest public health-based disease prevention programs. Since the emergence of NBS there have been no clear guidelines to explain testing to parents. Parents require information regarding NBS to minimize anxiety and improve compliance in the event of a positive screen (Newborn Screening Task Force). To examine how Minnesota parents are informed about blood spot and hearing screening, a phone/e-mail survey of 31 questions was conducted. The survey targeted the top 90% of birthing hospitals. Of 50 hospitals contacted, 35 responded (70%). We identified several differences in how hospitals provide information about blood spot and hearing screens. We found that for blood spot screening, 25 hospitals provided verbal information, 34 provided written information and 25 provided both. For hearing screening, 28 provided verbal information, 32 provided written information and 26 provided both. We also asked when informing occurred and found that 54% (verbal and written) informed prior to blood spot screen and 42% (verbal), 31% (written) informed prior to hearing screening. We reviewed copies of written information given to families and found that sometimes parents were given out-of-date materials lacking information on expanded NBS or referred to NBS as PKU screening. For hearing screening, we found parents were given a hospital made pamphlet providing up-to-date information. We also asked what was said and did not find evidence of consistent informing practices between hospitals. Through this survey, we identified areas that need improvement. We suggest that verbal information be scripted to ensure accurate conveyance of information and written information to be consistent and up-to-date, while informing parents about screening more than once using both verbal and written methods. Further educational efforts are needed to standardize the provision of newborn screening information to Minnesota parents.

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Cystic fibrosis newborn screening: a pilot study to maximize carrier screening. *E. Lagoe, S. LaBella, G. Arnold, P.T. Rowley.* University of Rochester, Rochester, NY.

Newborn screening for cystic fibrosis (CF) is expanding because early diagnosis results in improved nutrition and growth. Most newborns identified by a mutation panel have a single detected mutation and require sweat testing to exclude an additional undetected mutation. The resulting identification of CF carrier newborns, though not the primary purpose of screening, has three potential benefits, (1) the detection of trait x trait couples, (2) presymptomatic testing of these couples previously born children who may have undetected CF, and (3) a carrier parent alerting extended family members to the chance of being a CF carrier. Reaping each benefit requires genetic counseling and acceptance of carrier testing by parents. The purpose of this study was to utilize the sweat testing visit to educate parents about the value of carrier testing for themselves and their blood relatives. We compared *special care* (communicating their newborn's sweat test result, providing genetic counseling, and offering carrier testing to parents, all on the sweat test visit) vs. *standard care* (sweat test result reported by phone to the parent by the newborns physician the next day, ideally with the recommendation to arrange genetic counseling and parental carrier testing). In the first year of NYS CF screening, 64 newborns with one detected mutation were reported in the nine-county region that includes Rochester. Of these, parents of 39 agreed to participate in the study and to be randomized to *special* or *standard* care. Of 61 participating parents, 23 had carrier testing after the birth of the baby. The frequency of such parental testing was significantly higher in the *special* care group (17/34 or 50%) than in the *standard* care group (6/27 or 22%)(p0.05).

This is the first evidence from a randomized trial that parental genetic counseling and carrier test offered on the sweat test visit increases identification of carrier parents. Such identification detects trait x trait parents and facilitates carrier testing of relatives.

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Evaluation of informativeness of microsatellite markers for carrier testing of dystrophic epidermolysis bullosa in Tunisia. *H. Ouragini[1], W. Daoud[2], F. Cherif[2], S. Chakroun[1], C. Charfedine[1], S. Boubaker[3], A. Ben Osman-Dhahri[2], S. Abdelhak[1].* 1) Molecular Investigation of Genetic Orphan Diseases Institut Pasteur de Tunis, Tunis, Tunisia; 2) Dermatology department, Hôpital de La Rabta, Tunis, Tunisia; 3) Anatomo-Pathology service, Institut Pasteur de Tunis, Tunisia.

Dystrophic Epidermolysis Bullosa (DEB) is a clinically heterogenous blistering disorder of the skin and mucous membranes characterized by abnormalities in the anchoring fibrils (AF) and loss of dermal-epidermal adherence. It is inherited in either autosomal dominant or recessive mode. Mutations within the *COL7A1* gene (3p21) which encodes collagen VII, the major component of the AF, have been shown to be responsible for DEB. *COL7A1* is composed of 118 exons overlapping 32Kb. More than 200 mutations have been identified and most of them correspond to private mutations. Mutation screening is hampered by the size of the *COL7A1* gene and by the mutation heterogeneity. Analysis of polymorphic markers may provide a rapid and relatively easy method to identify carriers for genetic counselling. Our aim is to evaluate the informativeness of polymorphic markers for carrier testing of DEB in Tunisian families at risk. For this purpose, six consanguineous Tunisian families with at least one affected child have been analysed. Transmission of the disease within these families follows an autosomal recessive mode. All family members were genotyped with five microsatellite markers overlapping the *COL7A1* gene. The genetic map of this region is: cen-D3S1568, D3S3629, (*COL7A1*), D3S643, D3S1478, D3S3582-tel. For the two closest markers to *COL7A1*, D3S643 was fully informative in the six families and D3S3629 was fully informative in 5 families and partially in 1 family. Taking into consideration the informativeness and distance of these 2 markers to *COL7A1* (10Kb and 80Kb respectively), we propose the use of their use for carrier testing of DEB in North African population.

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High frequency of familial cardiovascular risk in a cancer genetics clinical population suggests the need for genetics services for adult-onset cardiovascular disorders. *A. Sturm, E. Varga, C. Eng.* Division of Human Genetics, The Ohio State University, Columbus, OH.

Cardiovascular disease (CVD) is the number one killer of men and women in the United States and other developed countries. Substantial evidence exists that genetic factors play a role in the risk for CVD. Previous studies have shown that up to 10% of individuals have a high familial risk and could benefit from risk factor modification, biochemical screening, early detection strategies, and genetic counseling. Family history has been shown to be a comprehensive and overall accurate initial method for the identification of high-risk individuals. This study was undertaken to assess the number of individuals attending a cancer genetics clinic who were also at high familial risk for coronary artery disease and/or thrombosis. Genetic counselors received inservice training regarding the clinical presentation and risk factors associated with CVD and collected pedigree data during the initial consultations for patients seen in the Clinical Cancer Genetics Program at The Ohio State University over a six-month period. Following initial consultation, two adult cardiovascular genetic counselors independently reviewed family history data and assigned high familial risk status based on published criteria. The proportion of high familial risk individuals in this clinical population was determined. Preliminary data over two months indicates that 31% (12/39) of patients referred for clinical cancer genetics consultation are also at high familial risk for CVD. These individuals require more aggressive management, including the assessment of biochemical and genetic risk factors and markers, the potential for early detection of subclinical disease, and the provision of a personalized prevention plan. Genetics professionals in various subspecialties who routinely obtain family history information will identify high risk for CVD in a considerable proportion of their patients. Because the opportunity for prevention of CVD in high risk individuals exists, the number of cardiovascular genetics programs whose focus is prevention of adult-onset disease should increase.

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Knowledge of CanMEDS 2000 roles and competencies among medical genetics residents. *M. Capelli[1,2], G.E. Graham[1,2].* 1) Depts of Genetics, Pediatrics and Psychology, Children's Hospital of Eastern Ontario; 2) University of Ottawa, Ontario, Canada.

Medical Genetics has been a primary specialty of the Royal College of Physicians and Surgeons of Canada (RCPSC) since 1989 and requires 5 years of clinical training beyond medical school. Like all specialty programs, Medical Genetics adheres to a national framework approved by the Royal College in 1996. CanMEDS (Canadian Medical Education Directives for Specialties), explicitly describes 7 essential roles common to Canadian specialist physicians, each with their own set of specialty-specific competencies. Specialty programs are required to incorporate these roles and competencies into their accreditation standards, objectives of training, in-training evaluations and final exam blueprints. CanMEDS was divided into phases: Framework Development (1990-96), Pilot Projects (1996-97), Implementation (1997-2002) and Faculty Development (2003-). This framework is now extensively implemented in Canada and has attracted international attention from medical educators. We surveyed 20 of 22 (91%) English-speaking Canadian Medical Genetics residents outside the province of Quebec between March and May 2005. All were from programs that had implemented the CanMEDS framework. None were able to correctly expand the acronym CanMEDS. The percentage of residents able to name the individual CanMEDS roles was: Medical Expert (15%), Communicator (10%), Collaborator (10%), Manager (10%), Health Advocate (10%), Scholar (5%) and Professional (15%). On a 5-point Likert scale (1=none and 5=heavy), residents reported the following mean emphasis in their programs: Medical Expert (4.15), Communicator (3.8), Collaborator (3.45), Manager (2.75), Health Advocate (2.9), Scholar (3.4) and Professional (3.65). When provided with the CanMEDS roles, the percentage of residents able to correctly match descriptors with their respective roles varied with the descriptor: Medical Expert (35%-80%), Communicator (35%-45%), Collaborator (60%-75%), Manager (20%-85%), Health Advocate (75%-80%), Scholar (50%-75%) and Professional (70%-95%). Is CanMEDs truly changing postgraduate medical education?

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Video self-assessment in the acquisition of CanMEDS competencies in medical genetics. *G. Graham[1,2], M. Capelli[1,2].* 1) Departments of Genetics, Pediatrics and Psychology, Children's Hospital of Eastern Ontario; 2) University of Ottawa, Ontario, Canada.

Medical genetics residencies and clinical genetics fellowships focus on the acquisition of consultancy skills for a large variety of patient presentations, including those requiring prenatal counselling, cancer genetic counselling, the diagnosis and management of genetic syndromes (dysmorphology) and the diagnosis and management of inborn errors of metabolism. In Canada, the training of geneticists is almost exclusively based on traditional approaches. In both inpatient and outpatient clinic settings, all programs routinely use one-on-one teaching by a staff geneticist before, during and following a patient encounter. Practice OSCEs (Objective Structured Clinical Exams) with standardized patients are regularly used in four of the seven residency programs. Video recording is not regularly used in any program. A search of the PubMed database using various combinations of genetics, counselling, training and video produced no publications addressing the use of video recording in the training of genetics residents or fellows. Following REB approval and with informed consent, 20 of 22 Canadian medical genetics residents and clinical genetics fellows have agreed to participate in a research project involving a 30 minute video-recorded OSCE scenario using a single standardized patient and a single staff geneticist observer. Using a CanMEDS assessment tool, we are measuring concordance between the residents self-assessments before and after video review of their performance; concordance between residents self-assessments and those of the staff geneticist; and concordance between the assessments of the resident, standardized patient and staff geneticist. All residents and fellows are also completing pre-and post-OSCE surveys examining their attitudes and preferences regarding the use of OSCE scenarios, physician observers and videotaping in clinical training. Data collection will finish in July 2005 and data will be summarized using descriptive statistics. Concordance and pre-post data will be analyzed using Chi-square analysis or paired t-tests.

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Long-term follow-up of "Genetics in Primary Care", a faculty development intiative for primary care providers.

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The Genetics in Primary Care (GPC) project, a national faculty development initiative, offered train the trainer workshops for primary care providers (PCP) with teaching responsibilities, using case-based teaching modules. Between October 2000 and April 2001, 79 participants from 20 U.S. medical training programs participated in the workshops. In 2004-2005, follow-up interviews were conducted by phone with 27 individuals from 10 sites. Among PCP, all respondents had made changes to their teaching practices following their participation in GPC, and 86% also reported changes to their clinical practice. Over four years later, 55% report a greater interest in genetics than before GPC. The most appreciated aspect of the project was the interdisciplinary collaboration (55%). Most commonly cited difficulties were maintaining the level of enthusiasm over time (36%), finding time (27%) and convincing other PCP of the relevance of genetics in their practice (23%). All of the PCP respondents have used the GPC materials for teaching purposes. Among genetics experts, all reported making changes to their teaching practices since their involvement in GPC, but only 20% reported changes in their clinical practice. 60% had little or no contact with PCP before GPC, but all showed interest in working with PCP in the future. For the genetics experts, maintaining enthusiasm over time was the hardest part of the project (40%), and interdisciplinary collaboration the most interesting (80%). 60% of the genetics experts used the GPC materials for teaching purposes. Of all respondents, 22% spontaneously reported that their participation in GPC was the launching point to getting a new position or participating in other projects. All would recommend a similar project to their colleagues. GPC has had lasting effects on participants' teaching and clinical practices, and has encouraged interdisciplinary collaboration. Future efforts will need to address sustainability issues.
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Medical school use of "Genetics in Clinical Practice: A Team Approach". *W.A. Faucett[1,2], H. Smith[3], N. Lamb[1], D. Waggoner[4].* 1) Dept of Human Genetics, Emory Univ Sch of Med, Atlanta, GA; 2) Center for Disease Control (CDC)and ORISE, Atlanta, GA; 3) Un of Arkansas Med Schl Library, Little Rock, AK; 4) Dept of Human Genetics, Univ of Chicago, Chicago, IL.

Introduction:*Genetics in Clinical Practice: A Team Approach*is an interactive cd-rom/web educational tool originally designed to provide 10+ hours of genetic education for practicing physicians. Developed by Dartmouth Medical School and the CDC it simulates a clinical rotation in genetics using current medical conditions (CF, colon cancer, hemochromatosis, Fragile-X). This educational tool was incorporated into the curriculum of the University of Arkansas Medical School(UAMS), Emory University School of Medicine(EUSM) and the Pritzker School of Medicine at University of Chicago(PSMUC). **Methods:** At each school the program was introduced to first or second year students. UAMS students were required to complete one patient scenario followed by a 36 question survey. EUSM students completed 6 of the 7 patient scenarios over 4 weeks, with weekly assignments and small group discussion sessions, followed by an optional 7 question survey. PSUMC students completed one of the patient scenarios and a 5 question survey to receive credit. Use of the cd-rom consitituted 5% to 10% of the student's grades. **Results:**Surveys found that 95% of the PSMUC students felt the program was a useful part of the class. Similarly, 90% of the UAMS and 89% of the EUSM students found the "patient encounters" useful. 92% of the PSMUC students felt that the cases augmented their class learning. 81% of UAMS and 87% of EUSM students felt the program contained "just the right amount" of information. Over 60% of UAMS and EUSM students felt the material was relevant to practicing physicians and 79% of the UAMS and 71% of the EUMS students felt they learned more than or the same amount that they could have learned in a real clinical setting. 89% of the PSMUC students recommended using the program in future classes. **Conclusion:**This educational tool is highly rated by medical students and can be successfully utilized in both medical school and healthcare training programs to simulate patient experiences in a genetic context.

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Constructivist Learning Theory in Genetics Education. *K.U. King, J. DeLuca, R. D'Aoust, J. Clements.* Univ Rochester, Rochester, NY.

Background: Inclusion of genetics curriculum in health education is essential to the clinical care of individuals, families and communities throughout the lifespan. Cognitive learning takes place in social interactions; the quantity and quality of these interactions impact the acquisition of critical thinking. Application of this theory is pedagogically consistent with educational needs of adult-learners. Purpose: To explore the implementation of constructivist learning theory conceptualized as collaborative, problem-based-learning in genetics education. Intervention: For 60 students, we designed and implemented group-based, social and adult learning activities that incorporate relativism or value of learning, bridging knowledge from previous-to-new in directed-problem-based learning. We anticipated that students would acquire higher order thinking skills to further apply genetics knowledge in novel situations encountered in clinical practice through complex thinking and collaboration. Individual students explored one genetic entity in-depth and collaborated in group projects and later prioritized findings for group presentations. Half of the groups developed expertise in Mendelian entities and half developed expertise in multifactorial entities. Assessment of this strategy consisted of student and faculty appraisal of individual and group projects and compilation of formal course evaluations. Findings/Outcomes: Students reported overall satisfaction with individual and group-based learning activities; they expressed that the groups were too large, and that they would have benefited from exploring more than one entity. Because students represent diverse backgrounds, they expressed satisfaction with the mutual development of skills as compared to traditional unidimensional learning. Faculty reported satisfaction with the process and outcomes of group learning projects. Interpretation/Discussion: Implementation of constructivist learning theory is a novel approach to genetics education. More follow-up is needed in to determine the application and generalizability of complex social and collaborative learning in clinical settings post-graduation as well as the specific application of genetics knowledge and principles to clinical care.

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Sharing Cx26/Cx30 genetic test information with family members. *T. Blase[1], A. Martinez[2], W.W. Grody[2], L.A. Schimmenti[3], C.G. Palmer[2].* 1) California State University Northridge, Northridge, CA; 2) University of California Los Angeles, Los Angeles, CA; 3) University of Minnesota, Minneapolis, MN.

Although clinical genetic testing for Connexin26/Connexin30 (Cx26/Cx30) is available, there is limited information regarding the sharing of test results and the effects of this genetic test information on families. A qualitative study was conducted to elucidate if, how, and why parents of children with hearing loss who have had Cx26/Cx30 genetic testing share the genetic test results with relatives. Parents of a child with hearing loss who had Cx26/Cx30 testing (n=7 positive results, n=4 negative results, n=1 inconclusive result) participated in a semi-structured interview. All participants shared their childs genetic test result with at least one relative. The reasons for sharing were diverse and differed as a function of the test result. *Duty to inform* was a common reason given by participants who received positive test results, but never voiced by participants who received a negative or inconclusive test result, suggesting that only those parents who receive positive Cx26/Cx30 test results feel that this information has a meaningful impact on their family members. Family members learning of a positive Cx26/Cx30 result were reported to express *surprise* while relatives learning of a negative test result were *unsettled* by this result. While a variety of reactions of relatives to the genetic test results were reported, relatives of individuals with positive test results expressed a wider range of emotions compared to those with negative or inconclusive test results. Similar to the disclosure process of genetic information to relatives for other conditions such as cystic fibrosis and hereditary breast/ovarian cancer, individuals whose child had Cx26/Cx30 genetic testing share genetic test information with close relatives, however, the etiologic heterogeneity of hearing loss affects the reasons for disclosure and responses of family members to Cx26/Cx30 test information. These differences suggest that hearing loss may have unique attributes that influence responses to genetic test information.

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Real Time PCR Strategy for Newborn Screening in Spinal Muscular Atrophy. *T.W. Prior, R.E. Pyatt.* Pathology, 121 Hamilton Hall, Ohio State Univ, Columbus, OH.

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by degeneration and loss of the anterior horn cells and associated progressive muscle weakness. 95% of affected individuals will have a homozygous deletion of exon 7 in the SMN1 gene. While a number of potential therapies are currently in clinical trials, their success may depend on identifying individuals as early as possible to begin treatment before potentially irreversible neuronal loss. Thus we have investigated the utilization of newborn blood spots for the early detection of SMA. Any potential screening protocols for SMA must demonstrate no overlap between affected individuals and carriers, utilize a minimum of target DNA, and most importantly distinguish the SMN1 gene from the SMN2 centromeric copy which has greater than 98% sequence homology. We have developed an assay for the detection of homozygous deletions in SMN1 exon 7 from neonatal blood spots using the Real Time PCR system. To specifically detect levels of SMN1, each reaction contained a competitive, non-extending oligo for SMN2 exon 7 in addition to a primer/probe set specific for SMN1 exon 7. 156 DNA samples extracted using a standard salting out method including 85 affecteds and 67 carriers were used to validate the assay specificity. Regardless of SMN2 copy number (0-5), normal individuals and carriers were distinguished from affected individuals in all cases based on amplification curves and cycle thresholds. Finally, we tested a series of 63 samples extracted from 50ul whole blood spots using a rapid purification protocol from Gentra which included 24 carriers and 16 affecteds. In all cases, affected samples were distinguished from carriers and normals regardless of their SMN2 copy number and with no amplification failures. Thus we have developed an automated and sensitive assay for the detection of SMN1 homozygous deletions with a potential application in newborn screening programs.

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One of six founder mutations account for nearly 75% of all BRCA1/2 mutations in French Canadian women attending two McGill University high-risk clinics. *K.V. Kotar[1,2,4], K. Osczevski[3], L. Yamani[1], M. Galvez[1,4], N. Hamel[1,4], E. Ferrara[1], P. Tonin[1,3,4], W.D. Foulkes[1,2,3,4].* 1) Div. Medical Genetics, Dept Medicine, McGill Univ. Health Centre, Montreal, QC, Canada; 2) Cancer Prevention Centre, Sir M.B.Davis-Jewish General Hospital, McGill Univ., Montreal, QC, Canada; 3) Dept Human Genetics, McGill Univ., Montreal, QC, Canada; 4) Research Institute, McGill Univ. Health Centre, Montreal, QC, Canada.

The identification of founder mutations in the French Canadian (FC) population of Quebec has simplified genetic testing. Oros et al (2004) found that in cancer-dense FC breast/ovarian cancer families (FCBOCF), one of five founder mutations accounted for 84% of all BRCA1/2 mutations. We set out to determine the proportion of BRCA1/2 mutations in a clinic population of FC women eligible for BRCA1/2 testing that are attributable to six identified founder mutations [BRCA1: (4446C>T, 2953del3+C); BRCA2: (8765delAG, 6085G>T, 6503delTT, 3398del5)]. FC probands were defined as francophone individuals having at least three grandparents born in Quebec. Of tested probands, 35/219 (15.9%) carried founder mutations [4446C>T (n=11), 2953del3+C (n=3); 8765delAG (n=14), 6085G>T (n=5), 6503delTT (n=1), 3398del5 (n=1)]. Two had non-founder BRCA1 mutations, (1135insA and 3875del4) identified serendipitously. Of 182 probands left, 125 were not tested further. The remaining 57 probands were sent to Myriad Genetics for further testing where nine (15.8%) were positive for a BRCA1 mutation (one each for 3875del4, 241C>T, 5641A>C, 4160delAG, two for 3773delTT, and three for 1061G>A), one had a BRCA2 mutation (2558insA) and 47 had no mutation detected. Thus 12/47 (25.5%) BRCA1/2 mutations were non-founder mutations. By haplotype analysis, the 1061G>A mutation has a common origin in the FC population but was not seen in other FCBOCF. As BRCA1/2 genomic deletions are rarely seen in this population (Moison et al, 2003), these 6 founder mutations account for ~75% of all BRCA1/2 mutations in the FC population. Hence, we conclude that pre-screening of all clinic-based FC patients for these six mutations is recommended prior to large-scale mutation analysis of both cancer susceptibility genes.

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Quality Management and Quality Assurance in Molecular Genetic Testing Laboratories. *E. Dequeker[1], S. Berwouts[1], J.J. Cassiman[1], M.A. Morris[2].* 1) Human Gen/Gasthuisberg O&N 6, Univ Leuven, Leuven, Belgium; 2) Medical Genetics Service, Geneva University Hospital, Switzerland.

Genetic testing services in Europe have substantially increased their activity in the past few years. Testing for genetic diseases has rapidly moved from the laboratory into medical practice and, in this process, issues of quality require adequate attention. The organization of annual external quality assessment schemes in Europe, complemented by regional workshops on quality, has demonstrated that quality assurance is essential in order to minimize errors in genetic services. It has been conclusively shown that errors and deficiencies can occur everywhere during the processes of sampling, genetic testing, interpretation, and reporting of results. The introduction of international standards for quality and competence in laboratories, together with a growing interest in accreditation of laboratories, has increased the need for a better understanding of quality management and quality assurance. The International Organization for Standards (ISO) defines quality as the degree to which a set of inherent characteristics fulfils requirements. The EUROGENTEST* network aims to help and guide medical genetic laboratories in order to better understand this type of standards language that is used by accreditation bodies. An educative website will be constructed with links for each EU country to relevant information on accreditation bodies, contact persons, coordinates of the national standardization institutes, and explanations of terminologies. Furthermore, a database will be prepared to publicize with relevant information on quality assurance in European laboratories. Finally, a study is planned to calculate the real cost for implementing and maintaining a quality management system in a genetic service laboratory. *EUROGENTEST is an EU project of 6th framework - Genetic Testing in Europe - Network for test development, harmonization, validation and standardization of services.

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Applicability and cost-effectiveness of the genetic test of adult-type hypolactasia in diagnosis of gastrointestinal symptoms in Finnish adults. *H. Rasinperä[1], S. Tikkakoski[2], A. Kotamies[3], H. Komu[1], I. Järvelä[1,4], K-L. Kolho[2].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; 3) Helsinki Center of Economic Research, Helsinki, Finland; 4) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland.

Gastrointestinal symptoms are common in everyday clinical practice. In this study we have evaluated the applicability and cost-effectiveness of the genetic test of adult-type hypolactasia by genotyping the single nucleotide polymorphism C/T_{-13910} associated with this trait. DNA of 1916 Finnish patients aged 18 to 64 years with or without gastrointestinal symptoms in outpatient clinics in capital area of Helsinki, were analysed by PCR-minisequencing. The participants filled in structured questionnaires concerning consumption of milk products and gastrointestinal problems. The frequency of the C/C_{-13910}-genotype associated with lactase non-persistence was 18%. Subjects with the C/C-genotype drank significantly less milk than those with the C/T- or T/T-genotypes associated with lactase persistence: 18% of the C/C-genotypes drank milk with meals compared to 38% and 36% of the C/T- and T/T-genotypes ($p< 0.01$). Based on preliminary analysis the C/C-genotype (84%) was related to gastrointestinal problems during the last three months more often than the C/T- or T/T-genotypes of lactase persistence (79% and 78 % respectively; $p< 0.05$). Bloating and flatulence was experienced by 53% and 79%, respectively, of those with the C/C-genotype (OR: 1.20 and 1.19, compared to the T/T-genotype). Moreover, our preliminary results in this population demonstrate that lactose tolerance test (LTT) in diagnosis of lactose malabsorption is even more inaccurate than earlier suggested. In conclusion, the genetic test for hypolactasia is a cost-effective method for primary screening of unspecific gastrointestinal symptoms in populations where the use of dairy products is common. The genetic test has been well accepted in the Finnish health care by the patients.

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Metylenetetrahydrofolate Reductase Polymorphism as maternal risk for Down Syndrome among Venezuelan Women. *W. Zabala, Z. Butron, S. Gonzalez, L. Borjas, MG. Portillo, K. Urdaneta, A. Morales-Machin.* Medical Genetic Unit, Univ Zulia, Maracaibo, Venezuela.

Down syndrome (DS) is the most commonly identified chromosome abnormality in humans attributed to the presence of three copies of chromosome 21 and is a major cause of pregnancy loss and mental retardation. Several studies indicated that the origin of the extra chromosome is maternal in about 80-90% of cases. Advanced maternal age is yet the only well-documented risk factor for nondisjunction, while the underlying mechanism remains unexplained. Recently, the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene has been related to maternal risk for DS. The hypothetical effect of C677T into maternal no-disjunction was liked to an altered DNA methylation pattern on the oocyte, secondary to reduced MTHFR activity of folate metabolism. In order to, determine the relation between the presence of the polymorphic variant 677T in MTHFR gene and the increased risk to have children with DS, we examined the C677T in 50 mothers of children with SD (MSD) and 50 mothers studied control (MC). Following DNA isolation, the MTHFR C677T polymorphism was analyzed by polymerase chain reaction and allele specific restriction digestion with Hinf I. Allele frequencies were calculated for each genotype, and the differences in allele frequencies between MSD and MC were determined using chi-square test. Odds ratios for both the heterozygous and homozygous mutant genotypes, was compared with the wild types, as a measure of association between the MTHFR genotypes and the maternal risk of SD. Genotype frequencies in the MSD were CC: 54%, CT: 34% and TT: 12%, and in the MC were 36%, 58% and 6% respectively, whereas the calculation of the relative risk (OR) was CC/CT OR: 0,39 [0,16- 0.90]; CC/TT OR: 1,33 [0.29 - 6.02]; CT + TT OR: 0,48 [0,21- 1.06]. The data presented in this study fail to support the relationship between MTHFR C677T polymorphisms and risk of having a child with DS.

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The complete reversion of a FRAXA premutation to a normal allele: implications for Fragile X genetic testing. *R. Polli, A. Casarin, L. Anesi, M. Martella, E. Leonardi, A. Murgia.* Dept Pediatrics, Univ Padua, Padua, Italy.

Fragile X Syndrome is the most common form of inherited mental retardation. The underlying molecular mechanism is the expansion of a series of (CGG)n repeats in the 5 untranslated region of the FMR1 gene. Expansions beyond 200 repeated elements are associated to methylation of the entire region, which is responsible of transcriptional silencing and loss of FMR1 protein. Rare cases have been reported in which the alteration was a point mutation or deletion of FMR1. Abnormal repeat expansions cause instability and lead to complex conditions of mosaicism in which Fragile X pre and full mutations, and even deletion/reversion patterns, are seen in the same individual. We report the case of a Fragile X family in which two independent events of complete reversion of a premutation to normal allele have occurred. A mother carrier of a Fragile X premutation with more than 70 CGG triplets, and a normal allele of 31 repeats was referred to our center for prenatal diagnosis in her second pregnancy. The molecular analysis was performed with PCR amplification and Southern blot on DNA on DNA from CVS sampling at the 13th week of gestation. The female fetus carried two normal X alleles: the paternal one with 24 repeats and a second one with 38 CGG trinucleotides; no evidence of mosaicism was detected. The first child, who was referred as mildly mentally retarded, was also tested. The Southern blot analysis showed a single fragment, corresponding to an apparently normal X allele and failed to detect the presence of other Fragile X expanded fragments. This allele was then characterized by PCR which obtained the amplification of a main product with 34 CGG repeats but also showed the presence of other, extremely underrepresented products suggesting a condition of instability in the Fragile X region. This finding has important consequences in the evaluation of appropriate strategies for Fragile X molecular analysis. The known frequency of somatic mosaicism, and the possible occurrence of complete reversions of FRAXA mutations strongly argue for a very careful evaluation even of normal-size alleles, particularly in Fragile X families.

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Identification of -galactosidase A mutations in females at-risk for Fabry disease: correlation of mutation status with clinical manifestations. *J. Wiszniewska[1], E. ORourke[2], K. Hoon[1], S.A. Darilek[1], A. James[1], M.J Darilek[1], J.A. Barranger[2], B.B. Roa[1], C.M. Eng[1].* 1) Dep of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Fabry disease (FD) is an X-linked lysosomal storage disorder caused by deficiency of -galactosidase A (-GalA), leading to progressive deposition of glycosphingolipids, primarily globotriaosylceramide (GL-3) in the vascular endothelium and cells throughout the body. The phenotype in heterozygous females ranges from being asymptomatic, to manifesting some symptoms with age, to females with clinical symptoms as severe as classic male patients. Identification of heterozygous females by enzymatic determination of -GalA activity is unreliable as the sole diagnostic test and current recommendations advise molecular testing for all at-risk females with a family history and/or symptoms compatible with the disorder. In a series of 92 females studied for GLA gene mutations, GLA mutations were identified in 30 of 65 (46%) females who were referred for DNA testing solely due to having a family history of the disease and 16 of 16 females (100%) who had a positive family history as well as reporting some symptoms consistent with FD. Enzymatic activity in these females fell into a broad range, from normal to severely decreased. There was no apparent correlation between degree of enzyme activity and clinical manifestations in these females. GLA mutations were found in three of eleven females (27%) referred for symptoms consistent with FD, but with no known family history. All three women with GLA mutations had decreased -GalA activity and renal manifestations, while the females without identified GLA mutations were referred for indications such as possible acroparesthesias and angiokeratoma. Our experience demonstrates the value of GLA mutation analysis in female patients for whom a diagnosis of Fabry disease is being considered. Presence of symptoms in females with a family history and renal manifestations in females with no family history were strong predictors of mutation status.

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Evaluation of a new microfluidics format for the detection of cystic fibrosis (CF) mutations using crudely prepared DNA lysates. *K.G. Monaghan, D. Bluhm.* Dept Medical Genetics, Henry Ford Hosp, Detroit, MI.

InPlex (Third Wave Technologies, Inc.), is a new method for CF mutation detection using multiplex Invader reactions. The assay involves target amplification of a DNA template, followed by standard Invader reactions performed within the channels of a plastic card. The assay tests for 41 CF mutations (including the 23 ACMG recommended mutations), I148T, F508C and the 5T, 7T and 9T alleles. The turn-around time from sample preparation to result is approximately 3.5 hours, with less than 30 minutes of hands-on time required to analyze up to 93 samples. To further decrease time and costs related to DNA extractions, we evaluated this assay using crudely prepared DNA lysates. The only difference in the method used for the crude lysates compared to highly purified DNA template was an increase in the number of amplification cycles from 14 to 15. Amplified DNA was then mixed with buffer and Cleavase, loaded onto an InPlex card and incubated. Cards were analyzed using a fluorescent plate reader. Samples were scored as either wild type, heterozygous, homozygous mutant or equivocal for each mutation analyzed. We ran a total of 87 lysates over the course of 3 independent assays, each with a normal control, positive control and no DNA control. Each lysate used was previously analyzed for CF mutations by another method. Of the 87 samples, 83 were negative for mutations previously screened (ACMG 25 plus 7 additional mutations). One sample was a known 3120+1GA heterozygote and 3 were known F508 carriers. All lysates derived from samples known to be positive for a CF mutation yielded the expected result. Of the 83 samples negative for the previously tested mutations, we identified 2 that were heterozygous for a mutation included in the InPlex assay (D1152H and D1270N) but not included in the original genotyping performed in the laboratory. Of the remaining 81 samples, 6 were called as equivocal and 3 yielded a low signal with uninterpretable results. There were no false positive or false negative calls using crude lysates with the InPlex assay. We are working to decrease the equivocal calls and low signals by adjusting cycling and incubation conditions.

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Identification of female carriers of DMD/BMD using real-time PCR and fluorescence gene dosage PCR. *E.K. Ra[1], M.W. Seong[1], S.I. Cho[1], S. Hong[1], J.Y. Kim[2], S.S. Park[1].* 1) Laboratory Medicine, Seoul National University Hospital, Seoul, Seoul, Korea; 2) Central Blood Laboratory Center, Korean Red Cross, Seoul, Korea.

Duchenne/Becker muscular dystrophy (DMD/BMD) is a severe X-linked myopathy, of which two thirds are caused by exon deletions within the dystrophin gene. Carrier detection is very important for the prevention of the disease, but deletion assay is affected by the presence of one normal X chromosome in the carrier. Although several methods such as Southern hybridization, quantitative PCR with radioisotope and FISH have been developed for carrier test, each has several disadvantages. We developed simple and rapid carrier test using real-time quantitative PCR and fluorescence gene dosage PCR, and performed on 14 female relatives (8 carriers and 6 non-carriers) of 10 DMD patients who had confirmed to have exon deletion. Dosage of deleted exon for each subject was determined by calculating ratio of deleted to non-deleted exon and followed by comparing this value to the ratio of normal control for both methods. Real-time PCR was done using primers and probes specific for each deleted or non-deleted exon and ABI Prism 7700 sequence detector. The fluorescence gene dosage PCR was carried out by 18-cycle amplification with fluorescence labeled primers and followed by analyzing on ABI 3100 genetic analyzer. Carrier status was accurately identified in all cases by both methods. The gene dosage range in carriers was 0.40 to 0.56 (0.450.05) with real-time PCR, 0.42 to 0.55 (0.500.05) with fluorescence gene dosage PCR, and, in non-carriers, 0.81 to 1.00 (0.910.08) with real-time PCR and 0.95 to 1.14 (1.020.07) with fluorescence gene dosage PCR. The coefficient variations of gene dosage determined by fluorescence gene dosage PCR were 2.4 to 4.1% in carriers and 2.4 to 5.9% in non-carriers. In conclusion, real-time PCR and fluorescence gene dosage PCR were simple, rapid and accurate method to identify female carriers of DMD/BMD and could be applied to determine gene dosage in other diseases with gene duplication or deletion.

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National CF Program in Chile: analysis of common CFTR mutations in 190 patients. *G.M. Repetto[1], A. Puga[1], M.L. Boza[2], M.A. Perez[3], O. Fielbaum[4], M. Betancourt[5], C. Pierry[6], M. Linares[7], M. Mendez[7], M. Oelcker[8], J. Silva[8], P. Astudillo[9], and members of the National CF program, Chile.* 1) Dept Genetics, Clinica Alemana-Univ Desarrollo, Santiago, Chile; 2) H. San Borja Arriaran; 3) H Exequiel Gonzalez Cortes; 4) H Luis Calvo Mackenna; 5) H Regional de Temuco; 6) H San Juan de Dios; 7) H Padre Hurtado y Sotero de Rio; 8) H. Regionales de Concepcion y Talcahuano; 9) Ministerio de Salud de Chile.

A National Cystic Fibrosis (CF) Program was initiated in Chile in 2000 to improve access to diagnosis and therapy for known patients in the country. Three hundred individuals have been registered, accounting for more than 90% of known patients. The Program has included access to molecular diagnosis for the registered patients since 2004. We report the findings of 191 unrelated patients with CF that were analyzed for the presence of 32 common mutations by PCR-OLA using Cystic fibrosis v3.0 ASR (Celera Diagnostics, USA). The overall detection rate was 40.1%, similar to other Latin American countries. Ten mutations were found: deltaF508 in 29% of alleles, G542X, R334W, 3849+10kb C>T, W1282X, R1162X with frequencies between 1 and 3% each, and G553X, G85E, 1078delT and G551D with less than 1% each. The polymorphic extragenic markers XV-2c and KM.19 were evaluated in twenty families of individuals with no mutation identified. There was no difference in haplotype distribution between the mutant alleles compared with the alleles not transmitted by the unaffected carrier parents. These results emphasize the need to improve the mutation detection rate in the country.

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Detection of glycine substitutions in the amino end of type I collagen by biochemical screening of fibroblast collagen requires supplementation by direct sequencing for osteogenesis imperfecta probands. *W.A. Cabral, S. Milgrom, E. Moriarty, J.C. Marini.* BEMB/NICHD/NIH, Bethesda, MD.

The biochemical test for osteogenesis imperfecta detects structural abnormalities in the helical region of type I collagen as delayed electrophoretic migration of alpha chains synthesized by cultured fibroblasts on SDS-Urea-PAGE. The sensitivity of this test is based on overmodification of alpha chains in helices with a substitution of an invariant glycine residue in a Gly-X-Y triplet. Substitutions delay helix folding locally, allowing excess glycosylation of OH-lysine residues. Biochemical testing is most sensitive for substitutions in the carboxyl end of a chain because helices fold in the carboxyl to amino direction. Although the standard biochemical test has been used for 2 decades, the limits of detectability are unreported. We compared the electrophoretic migration of normal fibroblast collagen from 30 patients with clinical OI (types III or IV) and known mutations in the amino half of the 1(I) and 2(I) chains of type I collagen and found that sensitivity differed for each chain. Sensitivity was enhanced on 5% rather than 6% PA gels, and by examination of intracellular as well as secreted collagen. In 1(I), substitutions in the first 100 residues were not electrophoretically detectable; 7% of cases in the current Mutation Consortium database are in this region. 1(I) substitutions between residues 100-230 were variably detectable while those after residue 232 were all detected. In 2, variability of electrophoretic detection extended through residue 436. About a third of cases in the Consortium database are located in the variable detection region. There was no correlation of substituting residues and biochemical sensitivity. Complete testing of probands with normal type I collagen biochemical results requires supplementation by direct sequencing of cDNA or gDNA in the amino regions of the alpha chains. Mutation detection in OI remains important for genetic counseling, reproductive decisions, exclusion of child abuse and genotype-phenotype correlations. In addition, identifying OI patients without collagen mutations will help define non-collagenous causes of OI.

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A new concept in mutation scanning. *R. Cotton[1,2], T. Tabone[1], G. Sallmann[1], E. Webb[1].* 1) Genomic Disorders Res Ctr, Fitzroy, VIC 3065, Australia; 2) The University of Melbourne, Parkville, VIC 3010, Australia.

We describe here a new approach for the detection of mismatched DNA in patient and control DNA samples. Mutation scanning is an important technique for research and diagnostic laboratories. Identification of unknown mutations has proved expensive by means of sequencing or laborious and less sensitive by means of alternative scanning techniques.

The detection of mutations as mismatched PCR amplicons is a well-established technique exploited by other mutation detection methods such as CCM and DHPLC. We have developed a simple mutation scanning method accurately and reproducibly detecting mismatched DNA in heteroduplex PCR amplicons without the use of toxic chemicals or separation techniques. The technology exploits potassium permanganate oxidation of mismatched bases to detect a mutation in a PCR amplicon, when present as a heteroduplex or by hybridising the test sample to a reference sample. The rate of modification is measured by the change in absorbance of the sample over time and compared against a known control. This assay is capable of analysing 96- to 384-well microplates in 2 hours. The initial set up cost of the assay requires a basic UV/Vis microplate reader and the ongoing cost for analysing each amplicon is less than AU$0.30 per sample.

Using this technique for genotyping, we have been able to successfully detect the 9 mutant samples in a set of 72 mitochondrial DNA samples from Melas syndrome patients using a range of PCR fragment sizes from 200-280 bp. In addition the assay, whilst not fully optimized for this application, has also proven 70% efficient in a blinded test to discover new mutations. This new approach to mutation scanning will provide a much needed alternative to the current and expensive mutation scanning methods and those suited to high-throughput applications that are not feasible for smaller diagnostic and research laboratories. The next phase will be to track-test the assay in collaboration with a diagnostic laboratory in order to evaluate the performance against currently established and more widely used mutation scanning methods.

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Study of MEFV mutations in the Iranian population by means of reverse-hybridization teststrips. *C. Oberkanins[1], S.H. Amini[2], B. Moghimi[3], A. Ghamari[4], N. Almadani[2], K. Kahrizi[4], G. Kriegshaeuser[1], H. Najmabadi[2,4].* 1) ViennaLab Labordiagnostika GmbH, Vienna, Austria; 2) Kariminejad/Najmabadi Genetic and Pathology Center, Tehran, Iran; 3) Medical School, Tehran University of Medical Sciences, Tehran, Iran; 4) Genetics Research Center, Social Welfare and Rehabilitation Sciences University, Tehran, Iran.

Familial Mediterranean Fever (FMF) is a hereditary inflammatory disorder caused by mutations in the MEFV gene. Carrier rates are known to be high among Turks, Armenians and Arab populations, whereas no data on the frequency and the spectrum of MEFV mutations were so far available from neighbouring Iran. We have applied reverse-hybridisation teststrips (FMF StripAssay) to simultaneously analyse twelve common MEFV mutations in 208 asymptomatic Iranians from different regions and ethnic groups. The overall frequency of mutant alleles in our study population (15.6%) was moderate compared to Armenia, but exceeded the values known from Turkey and Iraq. The most common variant E148Q was identified in 9.6% of MEFV genes. Five other mutations (P369S, M694V, V726A, A744S, R761H) were observed with lower prevalence. In addition, we studied the case of an 8 year old boy with short recurrent fever attacks and abdominal pain from a small village in the northwest of Iran. He turned out to be homozygous for MEFV mutation M694V. His parents were M694V/N and M694V/R761H. Several other members of this large family were found to be affected by typical symptoms of FMF and to carry MEFV mutations. Among 30 asymptomatic inhabitants of this village, who consented to participate in our study, we identified six different variants (E148Q, P369S, M680I(G/C), M694V, V726A, R761H) in a total of 13 mutant MEFV genes. Given the high frequency of MEFV mutations in Iran, the awareness for FMF and the availability of testing needs to increase significantly. (oberkanins@viennalab.co.at).

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Evolution of Ashkenazi Jewish Carrier Screening: Towards Comprehensive Reproductive Risk Assessment. *C. Oddoux, L. U, K. Hoang, N. Hayes, M. Banerjee, H. Ostrer.* Div Human Genetics, New York Univ Sch Medicine, New York, NY.

Recent advances in the understanding of the population genetics of Ashkenazi Jews (AJ) have identified increasing numbers of genes with founder mutations occurring at appreciable frequencies in this population. The genes include those encoding early-onset fatal and chronic diseases, late-onset diseases, and disabilities. This complexity represents a challenge to the development of consistent clinical services making use of this information and has led to the haphazard introduction of tests. We therefore applied similar strategies as those used by the ACMG for development of the policy statement on cystic fibrosis population-based carrier testing, to the development of a comprehensive carrier screening panel for AJ for implementation in our laboratory. Criteria for inclusion included: 1. Significant morbidity, whether severe or chronic; 2. Availability of an accepted intervention; 3. 100% penetrance; 4. Carrier frequency of 1% or more among AJ, 5. Mutation frequency of 1% or more among AJ affecteds. Tests for presymptomatic disease identification, or to screen for disabilities rather than diseases, were not included in the carrier testing panel. Based on these criteria, the following 16-disease AJ test panel was developed: cystic fibrosis, Canavan disease, Bloom syndrome , Fanconi anemia type C, Tay-Sachs disease, Gaucher disease, Niemann-Pick disease, familial dysautonomia, Glycogen storage disease, type 1A, Mucolipidosis, type 4, Usher syndrome, types 1 and 3, Familial hyperinsulinism, Maple syrup urine disease, Lipoamide dehydrogenase deficiency, and fragile X syndrome. In order to provide the benefits of multiplexing of tests while still allowing the patient the option of individual informed consent for each disease, a novel allele-specific oligonucleotide hybridization strategy was devised. The development of an extended AJ panel provided to the patient as individual tests provides the benefits of comprehensive carrier risk assessment without imposing testing for disorders the patient may not choose and eliminates the unopened window dilemma of the geneticist having access to information not available to the patient.

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Detection of African American and Hispanic Population-Specific Cystic Fibrosis Mutations in a Single-Tube Assay. *C.R. Novak, R. Saberi, J.T. Brown, A.G. Hadd.* Ambion Diagnostics, 2130 Woodward, Austin, TX 78744 USA.

PURPOSE: Molecular screening for cystic fibrosis mutations requires methods that are rapid, accurate and inexpensive. Improving risk assessment within ethnic populations is an important aspect of CF carrier screening. The purpose of our study was to develop a single-tube assay that incorporated Hispanic and African American population-specific alleles into the current ACMG recommended CF panel. METHODS: The assay combined single-tube multiplex PCR and a liquid bead array to query 90 alleles along the CFTR gene. Biotin-modified PCR products were directly hybridized to allele-specific capture probes, eliminating the need for secondary amplification or purification. Following the addition of fluorescent reporter, samples were analyzed for available genotypes in 15 seconds per patient using a Luminex platform. Results were automatically processed for allele ratio and genotype using Signature Script software. This data management software enabled selective revealing of ACMG recommended and ethnic specific alleles on a per patient basis. RESULTS: A single-tube multiplex PCR was optimized for 10-500 ng of input genomic DNA purified from whole blood. Using liquid bead-array hybridization followed by Luminex-based detection, a set of 23 CF mutations was expanded to 45 with detection of normal genomic DNA and respective mutant at 10 times signal-to-noise. In a direct-hybridization assay for 90 alleles, concordant mutation detection was demonstrated. The assay was completed in 4.5 hours with 50 minutes of hands on time (18% of total workflow). CONCLUSION: A multiplex PCR and direct-hybridization procedure was adapted for liquid bead array detection of an expanded CF carrier screening panel. Concordant results demonstrated that direct-hybridization screening assays could be modified to increase the number of alleles tested from a single patient sample. The addition of Hispanic and African American alleles as an adjunct to the current ACMG panel will provide expanded coverage to better address screening requirements for individual ethnic populations.

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The Genetic Testing Quality Control Materials Program (GTQC)- A sustainable community process to improve availability of appropriate, verified quality control (QC) materials for genetic testing. *L. Kalman, B. Chen, D.J. Boone.* NCHM, CDC, Atlanta, GA.

The expansion of molecular genetic testing in clinical and public health practice has increased the need for appropriate, verified quality control (QC) materials for quality assurance, test validation, proficiency testing (PT), and development of new genetic tests. However, despite the growing test volume and the rapidly increasing number of tests being offered, the necessary QC materials are not available for many tests.

The Centers for Disease Control and Prevention (CDC), in collaboration with members of the genetic testing community, has developed a program to improve public availability of QC materials and facilitate information exchange and communication on QC materials development, contribution, verification, distribution, and needs assessment. This CDC based Genetic Testing Quality Control Materials Program (GTQC) will provide continuing support and coordination to improve QC material availability. The GTQC will 1) facilitate the identification, procurement, development, verification and distribution of needed QC materials; 2) facilitate exchange of QC-related information; and 3) explore collaborative efforts for ongoing needs monitoring and materials development.

The GTQC is currently developing cell lines and genomic DNA QC materials for fragile X, Huntington disease, cystic fibrosis and disorders on the Ashkenazi Jewish Panel (Bloom, f. dysautononia, Canavan, Niemann-Pick, Tay-Sachs, Gaucher, Glycogen storage). More information about the program and available QC materials is available at the GTQC website, http://www.phppo.cdc.gov/dls/genetics/qcmaterials/default.aspx.

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Exploring community views regarding population fragile X carrier testing in a non-pregnancy health setting. *S. Metcalfe[1,2], A. Henry[1], S. Wake[3], L. Sheffield[3], J. Cohen[4].* 1) Genetics Education, MCRI, Royal Children's Hosp, Parkville, Australia; 2) Dept Paediatrics, The University of Melbourne, Parkville, Australia; 3) Genetic Health Services Victoria, Royal Children's Hospital, Parkville, Australia; 4) Fragile X Alliance Clinic, N. Caulfield, Australia.

Fragile X is the most common familial cause of intellectual disability. Since the identification of the gene in 1991 it has been possible to offer accurate carrier testing for fragile X. To date in, Australia, carrier testing has been confined to individuals with a family history of fragile X, however, testing at a population-wide level has not been explored. We have conducted focus groups of staff and female clients of a family planning clinic (a publicly-funded womens health care organisation) in Victoria, Australia, to assess their interest and concerns about offering population-based testing for fragile X carriers in this setting. In general, both staff and the clients were positive about the possibility of offering the program, although there were some concerns: the importance of up-skilling staff regarding counselling in offering the test; ensuring that women receive appropriate education prior to being tested and that genetic counselling be available for test-positive women; and that cost should not be a prohibitive factor. Staff were also concerned that offering such testing in a pre-pregnancy consultation was not the most ideal time, but this view was not echoed by the women. Information obtained from the focus groups are now being used to inform the development of a testing protocol and educational resources, for a pilot study to assess the feasibility and acceptability of offering population-based testing for fragile X carrier females.

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Multiplex PAP: Application to the detection of heterozygous deletions. *V.Q. Nguyen, Q. Liu, X. Li, S.S. Sommer.*
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Large heterozygous chromosomal deletions and gene duplications are important classes of mutations that are generally missed by standard PCR amplification and sequencing. Multiplex dosage pyrophosphorolysis-activated polymerization (MD-PAP), a derivative of PAP, was utilized to detect these types of mutations. PAP is a method for nucleic acid amplification in which 3 blocked oligonucleotides (P*) are activated by pyrophosphorolysis when annealed to the target template and subsequently extended. A key advantage to this technology is that PAP reactions produce little or no primer-dimer or false priming. As a result of this enhanced specificity, MD-PAP is easy to optimize. Herein, we utilize MD-PAP to determine gene dosage of each exon of the human factor IX gene by comparison with one endogenous internal control from the ATM gene. Estimated dosage is proportional to the actual template copy number over a minimum dynamic range from one to sixteen copies. A blinded analysis detected 100% of 43 heterozygous deletions of exons in the human factor IX gene.

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Pseudomonas infection in a Delta F508/I148T compound heterozygote negative for the 3199del 6 mutation; Implications for predicting CF phenotype in the prenatal genetic counseling setting. *J. Follmer.* Genzyme Genetics, Weston, FL.

A forty-year-old, gravida 1 para 0 presented for genetic counseling due to AMA. She and her husband were also Delta F508 and I148T (-3199del 6) CF mutation carriers, respectively. Amniocentesis revealed a normal male karyotype positive for both Delta F508 and I148T. Based on a limited number of case reports, this couple was counseled that classical CF symptoms were not expected when the I148T mutation (-3199del 6) is present in conjunction with a severe (i.e., Delta F508) mutation. It was also explained that non-classical symptoms of CF, however could not be ruled out due to lack of available data regarding the genotype/phenotype correlation of the I148T mutation. At six months of age this infant male presented to a pediatric pulmonologist because of a persistent cough. Sweat chloride tests had previously been negative. Pneumonia was ruled out, however a sputum culture was positive for *Pseudomonas aeruginosa*. To date, this male is one-year-old and thriving. He has no clinical manifestations of CF, however pancreatic function tests have not been performed. *Pseudomonas aeruginosa* is a gram-negative respiratory tract bacterium that is present in the lungs of 70% of CF patients and is one of the most serious complications of CF. It is a rare cause of infection in healthy individuals. No other case reports of Delta F508/I148T compound heterozygotes who have tested positive for *Pseudomonas aeruginosa* have been located.

CONCLUSION: This case study challenges the recent suggestions that the I148T mutation (-3199del 6) is only a benign polymorphism. Several studies have also suggested that the I148T be removed from genetic testing panels. However, this report emphasizes the complexity of providing accurate genetic counseling and raises the possibility of non-classical CF manifestations for I148T compound heterozygotes.

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Classic Congenital Myopathies Presenting with Lethal Neonatal Phenotypes. *L. Medne[1], J. Golden[1], R. Finkel[1], J. Ming[1], K. Ciprero[1], J. Lunardi[2], N. Monnier[2], C. Wallgren-Petterson[3], C. Bonnemann[1].* 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Centre Hospitalier Universitaire De Grenoble, Grenoble, France; 3) Haartman Institute, University of Helsinki, Helsinki, Finland.

We report two cases of classic congenital myopathies that presented with an unusually severe phenotype at birth and novel clinical features. Patient 1 presented with decreased fetal movements, IUGR, generalized severe hypotonia, distal arthrogryposis, fractures and respiratory distress requiring mechanical ventilation. Central cause of hypotonia was ruled out. Respiratory support was withdrawn at 24 days of age. Post-mortem muscle biopsy was consistent with central core disease (CCD). Compound heterozygosity of the Asn759Asp and Phe4808del mutations in the *RYR1* gene was found. Both asymptomatic parents were identified as carriers. Patient 2 presented with decreased fetal movement, polyhydramnios, severe hypotonia, fractures and poor respiratory effort requiring ventilation at birth. He was found to have absent suck and swallow, virtual lack of any movement and complete ophthalmoplegia. Family history showed that the parents were consanguineous; half first cousins from Ecuador. Muscle biopsy was consistent with nemaline myopathy (NM). Ventilatory support was withdrawn given the grave prognosis. *ACTA1* sequencing did not identify any mutations. Nebulin gene (*NEB*) sequencing is in process. Classic congenital myopathies are important differential diagnoses in neonatal akinesia. This is the second report of a rare lethal case of CCD, typically a non-life threatening autosomal dominant myopathy. While both of the *RYR1* mutations are novel, Phe4808del occurs in the functionally important and highly conserved transmembrane domain (seen in autosomal dominant CCD) of the calcium channel and Asn759Asp is in the highly conserved N-terminal domain. Autosomal recessive cases of severe NM have been reported; however, none have had complete ophthalmoplegia as observed in patient 2. Muscle biopsy was crucial in establishing the correct diagnosis in both cases allowing for appropriate genetic counseling.

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Diverse single- and multi-exon NF1 copy number changes represent two percent of the germ-line mutations in neurofibromatosis type 1 patients. *K. Wimmer[1, 2], S. Yao[1], K. Claes[3], H. Kehrer-Sawatzki[4], S. Tinschert[5], T. De Raedt[6], E. Legius[6], T. Callens[1, 3], H. Beiglböck[2], O. Maertens[3], L. Messiaen[1, 3].* 1) Laboratory Medical Genomics, Dept Genetics UAB,USA; 2) Abteilung für Humangenetik, Medizinische Universität Wien, Vienna, Austria; 3) Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 4) Dept of Human Genetics, University of Ulm, Germany; 5) Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus Universität, Dresden, Germany; 6) Centre for Human Genetics, Catholic University Leuven, Belgium.

Comprehensive mutation analysis achieves mutation detection rates of approximately 95% in NF1 patients fulfilling the NIH criteria. The vast majority of mutations are minor lesions and ~5% are total gene deletions. We found 13 single and/or multi-exon deletions/duplications using our RNA-based approach in a cohort of 1100 NF1 patients. We confirmed these copy number changes using a recently developed technique: multiplex ligation-dependent probe amplification (MLPA). Using MLPA, we further analyzed 55 NF1 patients for which thorough analysis was unable to uncover a pathogenic NF1 mutation. A novel multi-exon deletion/duplication was found in 12 of them. Thus, we have identified and/or confirmed by MLPA a total of 25 single- and/or multi-exon deletions/duplications. At least 19 of these deletions/duplications were unique. The extent of the 22 deletions and 3 duplications varied greatly and there was no clustering of breakpoints. We also evaluated the sensitivity of MLPA to identify multi-exon deletions present in a mosaic state and tested the ability MLPA P122-NF1 area assay, designed to identify total gene deletions, to distinguish between type I and type II deletions. Our study shows that intragenic deletions and duplications represent ~2% of all NF1 mutations. Due to this low percentage these copy number changes MLPA is not substantially increasing the mutation detection rate in NF1 patients. However, it may become a useful first step in a comprehensive mutation analysis scheme as it can quickly pinpoint patients with intragenic deletions/duplications as well as patients with total gene deletions.

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Performance of the Verigene System for multiplexed array-based mutation detection. *W. King, S. Haymond, T. Patno, W. Cork.* Applications Development, Nanosphere, Northbrook, IL.

The Verigene System (Nanosphere, Inc.) is a multiplex platform for direct detection of mutations and polymorphisms using unamplified human DNA. The patient sample is detected in a single-well microarray hybridization reaction. The method is based on hybridization of the target by allele-specific, surface-immobilized capture probes and oligonucleotide-functionalized gold nanoparticle probes, followed by a proprietary signal amplification process for target detection. The use of microarrays in molecular diagnostics allows multiplex detection of several genetic loci simultaneously in a single sample. It can be used to detect SNPs, and small insertions or deletions directly from genomic DNA. This platform is ideally suited for rapid detection of mutations without the complexities associated with PCR-based target amplification. In the present study, we assessed the analytical performance of the Verigene System for multiplex SNP detection of specific nucleotide sequences that are important for familial thrombophilia. The Factor V Leiden (1691 G->A, FVL) and Prothrombin (20210 G>A, PT) mutations were assessed in normal and mutant DNA. The following genomic DNA samples obtained from Sigma and Coriell Cell Repositories were investigated: Wild Type, homozygous PT mutant, homozygous FVL mutant and heterozygous PT and FVL. The Verigene System processed each of the samples and determined a genotype for each. The System demonstrated a 100% call accuracy and a 92.5% call rate. In additional studies aimed at measuring the reproducibility of call accuracy, 53/53 DNA samples isolated from peripheral blood of 9 normal donors were detected as WT PT (n=27) or WT FVL (n=26). In a similar set of experiments, feasibility was demonstrated for 24 of the ACMG recommended mutations for CF carrier screening. The CF assay results indicate that the Verigene System is capable of true multiplex mutation detection beyond single base changes. This low-complexity Verigene System array-based nanoparticle technology is also adaptable for use in a large variety of other clinical applications.

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Identification of a 45 X; 46 XX mosaic DMD carrier. *J.S. Parboosingh[1], T. Gillan[1], J.M. Miller[1], L.M. Graham[1], C. Davies[1], S. Merchant[2], A.M. Innes[1], S. Bamforth[2], J. Chernos[1], P.J. Bridge[1].* 1) Dept. Medical Genetics, Alberta Children's Hospital and University of Calgary, Calgary, AB, Canada; 2) Dept. Medical Genetics, University of Alberta, Edmonton, AB, Canada.

In 1988, a year after identification of the dystrophin gene, carrier testing was performed on a woman with a deceased affected son and her two daughters; no DNA was available from the affected son. Southern hybridization using a cDNA probe indicated that the woman had a reduced signal approximately equivalent to the signal strength of a male and that her daughters had normal female dosage. The interpretation of the results was that the woman carried a deletion of the dystrophin gene but that the daughters had not inherited the deleted chromosome. Recently, a grandson was diagnosed with DMD. Current molecular testing, consisting of MLPA analysis, revealed the presence of a duplication of exons 3 - 8 in both the grandson and the daughter. MLPA analysis on the woman suggested mosaicism for the duplication. Genotyping of X-linked markers revealed apparent homozygosity with apparent lack of transmission of a portion of the X chromosome to one daughter. Upon closer analysis of the genotyping data, the apparently non-transmitted alleles were present at very low levels. This data, together with the original Southern hybridization data, suggested that the woman was a mosaic with a major 45,X cell line lacking the DMD duplication and minor 46,XX cell line which carried the DMD duplication. This was confirmed molecularly by MLPA using a subtelomeric kit and the MECP2 kit for X chromosome dosage and cytogenetically; karyotyping revealed that 45/50 cells were 45,X and 5/50 cells 46,XX. Thus, what was originally interpreted as a dystrophin deletion is explained by the mosaicism for monosomy X. The degree of mosaicism observed in blood and buccal cells will be compared. This case illustrates the need to revisit molecular results in DMD families where carrier testing was performed in the absence of an affected, as well as, the power of molecular testing to detect mosaicism.

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Mutation detection in the *ATM* gene by haplotype analysis and Denaturing High Performance Liquid Chromatography (DHPLC) in A-T Italian patients. *S. Cavalieri[1], R. Gatti[2], P. Pappi[3], A. Funaro[1], N. Migone[1,3], A. Brusco[1,3].* 1) Genetics Biol & Biochem, Univ Torino, Torino, Italy; 2) Department of Pathology and Laboratory Medicine, The David Geffen School of Medicine at UCLA, Los Angeles, California, USA; 3) Medical Genetics Unit, San Giovanni Battista Hospital, Torino, Italy.

The mutation screening of the *ATM* gene in A-T patients is hampered by the large size of the transcript (9 Kb), the high number of exons, and the lack of mutational hot spots. In Iberian, Polish, Russian, and Brazilian populations SNPs and STRs haplotypes within ATM gene have been shown to be helpful in identifying recurrent mutations. Twelve Italian A-T patients (10 unrelated and 2 sisters), lacking ATM protein at Western blot, were pre-screened by SNP/STR haplotype analysis: we identified 3 homozygotes (2 sisters and one patient) and 9 heterozygotes. Three haplotypes were associated to known mutations: 3576GA mutation recurred in three patients and the two sisters; 7517_7520delGAGA was found in two patients (one heterozygote and one homozygote); 2250GA mutation was found in a single heterozygote. All are mutations already reported in Italian patients. A total of nine mutated alleles out of 22 were identified by SNP/STR analysis (40.9%). By DHPLC screening of the coding exons of the gene we detected additional 13/22 different mutated alleles. Eight of them are novel mutations. These combined techniques enhanced the efficiency of the mutation screening to a detection rate of 91% (20/22). Among the micro-mutations identified, 5 are nonsense, 5 cause a frameshift due to small insertions or deletions, 2 are missense(s) and four affect splice sites. A large deletion spanning exons 32-36 was identified in one patient by cDNA analysis and Southern Blot. This study confirms the importance of SNP/STR haplotyping to improve ATM mutation analysis, and shows that mutations missed by DHPLC may involve large genomic rearrangements in the ATM gene.

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Mutations in the ectodysplasin-A receptor gene *EDAR* in dominant and recessive hypohidrotic ectodermal dysplasia. *A.H. van der Hout, G.G. Oudesluijs, A. Venema, B. Mol, A.J. van Essen, Y.J. Vos.* Dept Clinical Genetics, University Medical Centre Groningen, The Netherlands.

Hypohidrotic ectodermal dysplasia (HED) is a congenital disorder characterized by the absence or hypoplasia of hair, teeth, and eccrine sweat glands. HED usually shows X-linked recessive inheritance but in a minority of cases it is inherited as an autosomal dominant or recessive trait. The X-linked form is caused by mutations in the ectodysplasin-A gene *ED1*. Boys are severely affected, women can show only some missing or malformed teeth and thin hair. Mutations in the ectodysplasin-A receptor gene *EDAR* were found in autosomal and recessive HED, one autosomal recessive case was described with mutations in the EDAR-Associated Death Domain gene *EDARADD*. We analyzed *ED1* in 38 independent patients as part of genetic counselling in their families. A causative mutation was found in 21 cases (55%). In the remaining 17 cases also *EDAR* and *EDARADD* were analyzed. Mutations in *EDAR* were found in five families (29%), no mutations were found in *EDARADD*. In four of these families which all show an autosomal dominant inheritance pattern the mutations, three nonsense mutations and one missense, are located in the Death Domain in the 3 end of the gene, supposed to be involved in protein-protein interactions. The phenotype in carriers of these dominant mutations is milder than in boys with a mutation in *ED1*. In the fifth family two affected boys were compound heterozygous for two missense mutations in a more 5 part of the gene. They have a phenotype comparable to X-linked cases. Both parents each carried one of the mutations. The father is unaffected, the mother, however, is affected but less severe than her sons. Until now only six families with mutations in *EDAR* were published, four with recessive inheritance and two dominant. Three of the five different mutations we found were also found in these studies. Therefore, although the numbers are small, the spectrum of mutations in *EDAR* seems limited. In all cases of HED negative for a mutation in *ED1* analysis of *EDAR* should be considerd, even though the pedigree is totally compatible with X-linked inheritance.

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A large pedigree with mitochondrial 1555AG mutation: Genetic counseling and point-of-care genetic testing by CASSOH for prevention of aminoglycoside-induced deafness. *S. Kure[1], J. Kanno[1], F. Kamada[1], T. Niihori[1], Y. Narumi[1], Y. Aoki[1], M. Hiratsuka[2], Y. Matsubara[1].* 1) Dept Medical Genetics, Tohoku Univ Sch Medicine, Sendai, Japan; 2) Dept Clinical Pharmacoceutics, Tohoku Pharmaceutical Univ, Sendai, Japan.

Recent studies in pharmacogentics have revealed various genetic polymorphisms that regulate drug responses. Individualization of the drug treatment based on this information would potentially maximize the effectiveness of medication and minimize its risks. Clinical application of the genetic information to individual healthcare has been hampered by a lack of a simple point-of-care genetic test that can be performed in an outpatient clinic or at bedside. Recently, we have developed a simple and raipd genotyping method, CASSOH, which utilizes immunochromatographic test strips. CASSOH method allows us to determine SNP within two hours (Matsubara and Kure, Hum Mutat; 2003:22:166, Hiratsuka et al, Drug Metab Pharmacokinet, 2004;19:303). We encountered a large deaf pedigree with maternal inheritance. Since streptomycine had been used for treatment of bacterial infections in deaf family members, we suspected that hearing loss was caused by mitochondrial 1555A>G mutation. The analysis of mitochondrial 1555A>G mutation by the CASSOH method revealed that two asymptomatic individuals were positive for the 1555A>G mutation. Additional four family members at risk were also tested. For prevention of further exposure to aminoglycoside antibiotics we issued the mutation-positive individuals with a medical alert card to warn doctors of the risk for hearing loss by aminoglycoside administration. The present case indicates that pharmacogenetic evaluation of clinically valuable SNP at genetic clinics would facilitate personalized medicine.

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Fragile X testing: Is it indicated in cases of premature ovarian failure? *M. Thangavelu, S. Bhatt.* Genzyme Genetics, Orange, CA.

Premature ovarian failure (POF) occurs in approximately 1% of women and is characterized by amenorrhea and elevated levels of serum gonadotropins before age 40. Etiologically POF is heterogeneous with involvement of different environmental and genetic factors. X-linked genes have been hypothesized to play a role in POF, one of them being the fragile X (FRAXA) gene. In particular POF is seen in carriers of the FRAXA premutation. Literature suggests that among fragile X premutation carriers 16%-33% experienced POF and in two fragile X families, 60% and 67% of premutation carriers experienced POF. On the other hand, among females experiencing POF only 4.1% to 6.8% were found to be carriers of the FRAXA premutation. Fragile X carrier testing results on 108 specimens received in our lab for the specific indication of POF were reviewed retrospectively. Of the 108 samples, 4.6% of patients (n= 5), were identified to have a premutation in the FRAXA gene, with repeats ranging from 64 to 80. Interestingly, 2 patients were identified to have alleles in the gray zone with 47 and 52 repeats. In an additional patient, only one normal allele was identified suggesting presence of only one X chromosome (possibly a 45,X karyotype). The incidence of permutation carriers in this cohort of patients with POF is similar to that previously observed. Interestingly all our patients identified to have premutation were referred from infertility practices. In view of the association between POF and fragile X, it is important that evaluation for fragile X premutation is part of the laboratory work up for POF. Although the clinical significance of observations in the grey zone is as yet unclear from the perspective of the classic fragile X syndrome, the observations in this cohort suggests the need for further evaluation for possible correlation with POF. Based on the emerging data on the association between POF and fragile X premutation, it is important that in addition to other clinical facets of fragile X syndrome, implications of POF and related fertility issues are discussed with the premutation carriers.

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Educational priorities in genetics for GPs, Paediatricians and Gynaeco-Obstetricians in 5 European countries: the Gened survey. *C. Julian-Reynier[1], JM. Calefato[1], I. Nippert[2], H. Harris[3], AM. Plass[4], L. Ten Kate[4], U. Kristofferson[5], J. Schmidtke[2], C. Benjamin[3], K. Challen[3], R. Harris[3], and the GenEd group.* 1) INSERM UMR379, Inst Paoli-Calmettes, Marseille, France; 2) Institut Fuer Humangenetik, Universities of Muenster and Hannover, Germany; 3) GenEd co-ordinating centre, Department of medicine, Manchester University, Manchester, UK; 4) VU Medical Centre, Amsterdam, Netherlands; 5) University Hospital, Lund, Sweden.

The extension of genetic knowledge to all fields of medicine involves progressively all primary care providers . The objective of this study was to measure how genetic educational needs were prioritised by the doctors practicing in 5 European countries. Methods : Questionnaires mailed to GPs, Gynaeco-Obstetricians and Paediatricians (France, Germany, Netherlands, Sweden, United Kingdom) in 2004 . Answer rate 41% (N=3686. Through a Component Principal Analysis, the 30 genetic educational priority items were reduced to 6 sub-dimensions. Results: The 3 first educational priorities for GPs were Genetics of Common Diseases, Risk assessment, and Psycho-social/counselling issues; whereas Basic genetics, Ethical Legal Public Health Issues (ELPHI), Techniques and innovations had lower scores. The ranking was consistent across the countries, but the scores differed ($p<0.01$) across the countries after multivariate adjustment. French GPs systematically quoted higher priorities for all 6 dimensions compared to other countries ($p<0.01$). The 3 first educational priorities for specialists were Basic Genetics/Congenital Malformations, Risk assessment, and Psycho-social/Counselling issues. ELPHI, Techniques/Innovations and Common Diseases were ranked in decreasing order afterwards. Ranking was consistent across the 5 countries but the scores differed significantly ($p<0.01$) across the countries after multivariate adjustment.The country effect was more heterogeneous than for GPs. Conclusion: Genetic educational priorities differ for GPs and specialists and across the countries. Interpretation should be done in according to the national health care systems and educational policies.

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The provision of a multidisciplinary screening, follow-up and research clinic for families with cardiomyopathy in Newfoundland. *Y. Teplitsky[1], P. Parfrey,[2], L. Kernaghan,[3], S. MacKay,[3], B. Peddle,[1], P. Ryan,[1], C. Tilley,[1], L. Thierfelder[4], A. Bassett,[5], T. Young,[2], W. McKenna,[6], S. Connors,[1], K. Hodgkinson.[2].* 1) Faculty of Medicine, Memorial University , St. John's, NL, Canada; 2) Patient Research Centre, Memorial University, St. John's, NL, Canada; 3) Clinical Genetics, Health Sciences Centre, St. John's, NL, Canada; 4) Max-Delbruck Centre, Berlin, Germany; 5) Clinical Genetics Research Program, CAMH, Toronto, ON, Canada; 6) The Heart Hospital, London, UK.

103 families with cardiomyopathy, with and without sudden cardiac death, have been ascertained in Newfoundland. Thirteen families have hypertrophic cardiomyopathy, 40 are part of the arrhythmogenic right ventricular cardiomyopathy (ARVC) spectrum, 34 have dilated cardiomyopathy and sixteen large families have ARVC linked to a disease associated haplotype at 3p25 (ARVD5). All are followed clinically through a genetics/cardiology clinic where at-risk individuals are offered genetic counselling and routine cardiac testing. Blood is taken for known mutation testing, haplotype testing or linkage analysis, whichever is appropriate, following informed consent. To date, 93 families have been seen and 318 subjects have had at least one visit. In 3p25 families, subjects found to have a high-risk haplotype are screened yearly until treatment is recommended. Subjects with a low-risk haplotype are followed every 2-3 years (to allow for error in the absence of a known mutation); those with an unknown haplotype status for 3p25 are screened every 1-2 years. The remaining families have clinical screening every 1-3 years. We have begun to screen for known ARVC/DCM/HOCM mutations. For families negative for all DNA screening, linkage analysis is considered. The clinic addresses both clinical and research issues in a synergistic manner. We aim to determine the genetic epidemiology including geneotype/phenotype analysis, assessment of genetic and environmental modifiers to the phenotype, and the natural history and clinical course of each cardiomyopathy. To aid this endeavour, an extensive patient brochure has been produced for patients, detailing both research and clinical issues.

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Family as Context in Clinical Genetics Project (FCCGP): an interactive, web-based curriculum, genetic information resource, and continuing education program for health care professionals (HCPs) and students. *C. Kenner[1], R. Parker[2], R. Fineman[2], S. Cummings[3], M. Michela[4], P. Bashook[2], L. Hruska[5], T. Swift-Scanlan[6], S. Olsen[6], A. Gallo[2], T. Savage[2], M. Knisley[5], S. Feetham[7], Y.T. Chang[8], A. Finkelman[9], S. Boyer[2], D. Siow[1].* 1) OU Nursing, OK City; 2) UIC Nursing; 3) U of Chicago Cancer Risk Clinic; 4) UIC Academic Affairs; 5) UIC Medicine; 6) Johns Hopkins U Nursing; 7) HRSA; 8) East Carolina U Medicine; 9) U of Cincinnati.

FCCGP is an interactive, web-based curriculum that aims to integrate new genetics information into medical, nursing and social work practice using the family as the context for care. This interdisciplinary project utilizes three case reports (including breast/ovarian cancer, cystic fibrosis and bipolar affective disorder) and offers learners an opportunity to integrate genetic, environmental, behavioral, psychosocial, ethical, legal and financial information into patient/family care. Core competencies from the National Coalition for Health Professional Education in Genetics and other sources were used to guide case report learning objectives, content development, and evaluation. The case reports, their pre- and post-test questions and answers, etc., have been reviewed and validated by practicing HCPs and can be found at : http://ce.nursing.ouhsc.edu/fccg/index.htm Integrating genetics and genetic services into the daily practice of HCPs continues to be one of the greatest challenges of professional genetics education. The FCCGP provides HCPs with a step-by-step process needed to deliver basic genetic services and/or referral of patients and families to genetic health care professionals. This how-to approach, coupled with downloadable tools and continuing education credits for physicians and nurses, provides practical information to HCPs, and encourages them to integrate genetics into their every-day practice. Lastly, barriers to implementing a successful, web-based curriculum, such as the FCCGP, will be described in order to help others who are interested in creating a similar online genetics education program. Supported by NIH Grant 1 R25 HG02259-01A1.

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Estimation of absolute risks of morbidity and mortality of disease given a family history. *A.S. Butterworth[1], P. Pharoah[1,2].* 1) Cambridge Genetics Knowledge Park, Cambridge, United Kingdom; 2) Department of Oncology, University of Cambridge, United Kingdom.

The counselling and management of individuals with a family history of disease is dependent on reliable estimates of absolute risk. Such estimates may also be useful for appropriate weighting by insurance underwriters. However most epidemiological studies present relative rather than absolute risks.

The aim of this study was to estimate absolute risks of morbidity and mortality for several common chronic diseases based on the available epidemiological data. Relative risks for different patterns of family history of colorectal cancer, prostate cancer, stroke and multiple sclerosis were obtained by pooling the results from all available studies using random effects meta-analysis. Relative risk estimates for breast and ovarian cancer were obtained from previously published meta-analyses. These relative risks were converted into absolute risks using actuarial life-tables, which use population incidence and mortality data and take into account competing risks of mortality.

For common cancers, the relative risk is approximately doubled for an individual with a first-degree relative (i.e. parent, sib or offspring) affected by the same cancer. The risk increases further with more affected relatives or a relative diagnosed at a younger age. However absolute risks vary substantially for different cancer sites. For example, a relative risk of 2.24 (95%CI 2.06-2.43) for having a first-degree relative affected with colorectal cancer is equivalent to a lifetime risk of 3.6% (~1 in 30) for a 30 year old compared to 2.0% (1 in 50) in the general population. Having a second affected first-degree relative increases the absolute risk to 7.4% (~1 in 14). The risks of dying from colorectal cancer by age 70 are 0.75% (~1 in 130), 1.4% (~1 in 70) and 4.1% (~1 in 24) for the general population, one affected relative and two affected relatives respectively. Similar results were found for the other diseases studied. These estimates should be useful for clinical risk assessment and may also inform insurance underwriting policies.

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Using family history of hemochromatosis or iron overload to aid targeted screening in a multiethnic, primary-care population: The Hemochromatosis and Iron Overload Screening (HEIRS) Study. *E.L. Harris[1], R.T. Acton[2], B.M. Snively[3], J.C. Barton[2], C. Leiendecker-Foster[4], C.E. McLaren[5], P.C. Adams[6], M. Speechley[6], T.C. Bent[5].* 1) Kaiser Permanente Center for Health Research, Portland, OR; 2) University of Alabama at Birmingham, AL; 3) Wake Forest University, Winston-Salem, NC; 4) University of Minnesota, Minneapolis, MN; 5) University of California, Irvine, CA; 6) London Health Sciences Centre, London, Ontario.

Screening family members of individuals with hereditary hemochromatosis (HH) is an approach to identify individuals at increased risk of developing clinical complications of iron overload (IO) who may benefit from preventive interventions. However, detailed family information is not always available to inform such an approach. Family history may be available. In the HEIRS Study, a multi-site, ethnically diverse, primary-care-based screening study for HH and IO, we enrolled 98,529 participants who did not report having been diagnosed with HH or IO and who were not recruited into the study by other family members. Here, we assess how strongly self-reported family history of HH or IO is associated with screening results that indicate increased risk of developing/having IO: *HFE* C282Y/C282Y (YY); or evidence of elevated body iron stores [men: transferrin saturation (TS) >50% and serum ferritin (SF) >300 g/L; women: TS>45% and SF>200 g/L].

Among 43,453 Caucasians, 1.18% of those having a family history of HH/IO were YY compared with 0.46% of those without an HH/IO family history [relative risk (RR)=2.55; 95% confidence interval (CI)=1.61-4.03]. Among 55,076 non-Caucasians, 0.17% with an HH/IO family history were YY compared with 0.022% of those without an HH/IO family history (RR=7.51; CI=1.68-33.5). Among Caucasians, 2.12% with an HH/IO family history had elevated TS and SF compared with 1.66% of those without an HH/IO family history (RR=1.27; CI=0.91-1.77). Among non-Caucasians, 1.42% with an HH/IO family history had elevated values compared with 2.25% of those without an HH/IO family history (RR=0.63; CI=0.39-1.02). These results indicate that self-reported family history of HH or IO may aid in identifying a high-risk group for *HFE* C282Y testing.

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BRCA1/2 Variants of Uncertain Significance: Assessment of Hypothetical Risk Management Recommendations.

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The main objective of presymptomatic genetic testing for hereditary cancer syndromes is to provide patients with specific risk information and tailored management recommendations. However, approximately 13% of Hereditary Breast and Ovarian Cancer testing reveals a variant of uncertain significance (VUS), an ambiguous result without established guidelines for clinical recommendations. Current VUS management is often based solely on the prior education and clinical experience of the health care provider, about which little is known. We surveyed 38 medical oncologists to assess their risk management recommendations for one hypothetical patient under 3 scenarios: 1)no genetic testing, 2)a *BRCA* mutation positive result, and 3)a VUS result. When asked a basic VUS definition question, 26.3% reported they were unsure of the meaning of a VUS. General screening, breast MRI, and surgical recommendations were significantly different between scenarios. With a VUS result, general American Cancer Society screening guidelines were less likely to be recommended than without genetic testing and more likely than with a positive HBOC result (18.9% v. 33.3% v. 5.5%; p=0.008). Breast MRI (32.4% v. 19.4%; p=0.001), breast ultrasound (16.2% v. 13.9%), ovarian cancer screening (48.6% v. 36.1%), tamoxifen (8.1% v. 2.8%) were all recommended at increased levels with a VUS result versus no genetic testing; all were most frequently recommended for patients with positive *BRCA* mutations. Respondents recommended oral contraceptives least frequently with a VUS and most frequently with positive status (5.4% v. 13.2%). Prophylactic breast and ovarian surgeries were only recommended in the positive genetic testing scenario (44.4% and 67.6% respectively). The reasoning behind all management recommendations also changed depending on the genetic testing status. A risk-based methodology was used least frequently with the VUS scenario. This study provides evidence that increased education regarding genetic testing, specifically variants of uncertain significance, is needed in the general oncology field.

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No gender differences observed in stability of fragile X CGG repeats in common and intermediate alleles. *S.L. Nolin, J. Silverman, A. Glicksman, S.Y. Li, T. Sukontasup, W.T. Brown.* NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

The CGG repeat within the FMR1 gene exhibits remarkable instability in families with fragile X. Sullivan et al. (AJHG 70:1532, 2002) examined 1452 parent-child transmissions of common (10-39 repeats) or intermediate (40-59 repeats) alleles and reported that unstable transmissions were seen more often in paternal than maternal transmissions. Furthermore, they observed a low level of unstable transmissions in common repeats sizes and higher rates in the intermediate alleles. We have examined 1216 parent-child transmissions of common and intermediate alleles and observed no unstable transmissions of alleles between 10 and 39 repeats. For alleles with 40-49 repeats, 9% (4/45) of maternally transmitted alleles were unstable as compared to 8% (2/26) of paternally transmitted alleles. For alleles with 50-59 repeats, 22% (13/59) of maternally transmitted alleles were unstable and 18% (2/11) paternally transmitted alleles were unstable. These studies indicated that in our sample there was no apparent gender bias in unstable transmissions of intermediate alleles and no unstable transmissions among common alleles. AGG analysis of the intermediate alleles in males revealed that nearly all unstable transmissions occurred in alleles with pure 40 CGG repeats. Specifically, 9/19 alleles with 40-49 pure repeats and 2/2 with 50 pure repeats exhibited unstable transmissions. In addition to these, one allele with 20 pure repeats increased by 1 repeat on transmission. Thus, AGG analysis suggested that ~50 pure repeats may be the threshold for consistent instability of FMR1 alleles.

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Validation of PCR Amplification and Capillary Electrophoresis for Accurate Sizing of Fragile X CGG Repeats.
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INTRODUCTON: Fragile X (FraX) premutation carriers are clinically unaffected with FraX Syndrome but females are at risk for passing expanded alleles to offspring. There is a linear correlation between the size of the premutation allele and risk for expansion; therefore, it is important to accurately determine the number of CGG repeats for individuals with alleles in the premutation range. MATERIALS AND METHODS: We employed a fluorescent PCR-based assay for detecting the number of CGG repeats in the FMR1 gene. The FraX CGG repeat region is PCR amplified using fluorescently labeled primers and products are separated by capillary electrophoresis (CE). Accuracy of sizing was determined by comparison to commercially available FraX controls. RESULTS: The FraX PCR assay was used to determine the number of CGG repeats in normal, intermediate, and premutation alleles. Ten commercially available samples were tested, three of which had repeat number verified by the GTQC Materials Program. Each sample was analyzed an average of 9 times with highly precise measurements in each replicate. The largest allele tested had 107 repeats and was consistently detected by the assay. Smaller repeats were sized accurately based on the length of known controls, whereas longer repeats gave results that varied more widely from the expected size. With the use of multiple controls of variable repeat length, we were able to accurately estimate the number of repeats to within 5 percent of the reported size. However, only 3 of the 10 samples we analyzed had repeat length verified and it is possible that the reported size of the remaining samples is inaccurate. CONCLUSIONS: We have validated a protocol for sizing of FraX CGG repeats for use in a clinical molecular laboratory. CE facilitates screening and sizing of patients with suspected FraX premutations. The PCR/CE-based FraX test is highly precise in the measurement of CGG repeats in the FMR1 gene. We found accurate sizing of alleles to vary depending on the size of the repeat. Additional control materials with repeat size verified by sequencing would greatly aid validation and quality assurance in laboratories performing this assay.

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MECP2 gene rearrangements in female and male patients with features of Rett syndrome. *P. Fang[1], P.A. Ward[1], S.A. Berry[3], M. Irons[4], B. Chong[1], I.B. Van den Veyver[1], J. Neul[2], A.K. Percy[5], D.G. Glaze[2], H.Y. Zoghbi[1,2], B.B. Roa[1].*
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About 86% female patients with Rett syndrome (RTT) have point mutations or small deletions/insertions in the MECP2 gene, which can be identified by DNA sequencing. In addition, large gene rearrangements have also been documented. We report data from our past 10 months of clinical testing for MECP2 mutations. We studied a subset of 256 female patients with well documented clinical diagnoses, including 216 classic and 40 atypical RTT cases. MECP2 sequencing identified mutations in 185 (86%) classic, and 25 (63%) atypical RTT patients. Southern analysis identified MECP2 gene rearrangements in 21 (~10%) classic and 1 (2.5%) atypical RTT patients. We also performed Southern analysis on 268 female and 65 male patients referred from various sources with limited clinical information. We found 8 female patients (3%) with partial gene deletions in this group. It is intriguing that we found a similar percentage of males with gene rearrangements - two males (3%) were identified to have duplications of the entire MECP2 gene. These cases involve a 9-year old boy and an unrelated 11.5-year old who presented with clinical features suggestive of Angelman syndrome (AS) and RTT. AS testing by either methylation or FISH analysis, and UBE3A gene sequencing were normal for both patients. MECP2 sequencing was normal, but Southern analysis showed duplication of MECP2 exons 1 through 4 in both cases. MECP2 gene duplication was verified by MLPA analysis in both patients. DNA from the 9-year old patient was further confirmed by chromosomal microarray analysis. The duplications appear to span common regions that include the L1CAM gene located ~200kb centromeric to MECP2. DNA sequencing followed by analysis for rearrangements in the MECP2 gene significantly increased the mutation detection rates for RTT, and further demonstrates the clinical value of MECP2 testing for female and male patients with a spectrum of related clinical phenotypes.

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Consumer Attitudes toward Genetic Testing and Newborn Screening for Hearing Loss. *A. Pandya[1], S. Burton[2], K. Withrow[1], A. Kalfoglou[3], S.H. Blanton[1], K.S. Arnos[2].* 1) Human Genetics, Virginia Commonwealth University Medical Campus, Richmond, VA; 2) Department of Biology, Gallaudet University, Washington DC; 3) NHGRI, NIH, Bethesda, MD.

The introduction of early hearing detection and intervention programs (EHDI) and the recent progress in identification of genes for deafness has lead to greater utilization of genetic services by parents of children identified with hearing loss (HL). However, efforts to assess consumer attitudes toward genetic testing for HL have lagged behind. We held five focus groups with 44 participants to explore their attitudes towards genetic technologies, motivation for seeking genetic testing, views about newborn hearing screening and reactions to the idea of adding molecular screening for HL at birth. Focus group participants included hearing parents of deaf children, Deaf parents and young Deaf adults of diverse ethnic backgrounds. Trained moderators presented a series of questions and the discussions were recorded and transcribed. A team of co-investigators reviewed the transcripts to identify major themes and developed a qualitative coding structure. The data were coded using the qualitative data analysis software NVivo 4.0. Perceived benefits of newborn screening and genetic testing for HL included helping parents 1)accept the diagnosis 2)share results with family members 3)anticipate the need for medical care, and 4)assess chance of having additional children with HL. Attitudes about the timing for providing genetic evaluation and testing were mixed; participants placed emphasis on respecting variations in the readiness of the family. Several parents expressed an interest in being offered molecular screening for common deafness genes early, potentially as an adjunct to audiologic newborn hearing screening. However, a recurring concern was the cost effectiveness & the need for parental consent. The results of these studies highlight the effectiveness of small focus group sessions to address complex and sensitive issues. The results from this analysis will guide the development of a comprehensive and sensitive survey instrument to be used on a national level.

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Population genetic research: Autonomy, human dignity and the evolution of informed consent norms in Canada in the post World War II era. *L. Sheremeta, T. Caulfield.* Health Law Institute, University of Alberta, Edmonton, Alberta, Canada.

Modern bioethical principles enunciated in the Nuremberg Code and the World Medical Association Declaration of Helsinki have served the research community and research subjects well for over fifty years. Founded on respect for human dignity, autonomy of the individual, a requirement for free and informed consent of research subjects and review of all human subject research by a research ethics board, these principles are intended to ensure that only ethically defensible and scientifically justified research is undertaken. Increasingly, the application of these principles to genomic research, which depends on the collection, storage and use of human biologic samples, is challenged. Small and large-scale biobanking projects throughout the world are struggling to deal with how consent principles impact the feasibility of population genetic research. UK Biobank, the Icelandic Health Sector Database, the Estonian Genome Project and the proposed Canadian Lifelong Health Initiative are examples of large-scale projects in which these issues have arisen. In Canada, strict interpretation of ethical guidelines and consent law preclude the use of human biologic samples without informed consent in studies where the samples are persistently linked to health information about the research subject. In the context of research involving previously stored human biologic materials this limitation is vexing to researchers. In the context of prospective research involving human biological materials the concept of informed consent demands that consent be voluntary and specifically referable to a defined research project. Given that researchers cannot predict all future research uses to which HBMs may be put, it is legally uncertain whether consent to such research can ever be informed. It has been suggested that ethical norms and the law should be weakened to facilitate research of this kind. This paper will enunciate the arguments for and against such normative evolution and will discuss the feasibility of various consent options that have emerged.

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Community-based medical home for patients with complex genetic disorders- a model to follow? *H. Wang[1], L. Nye[1], J. Renner[1], J. Tumbush[1], H. Morton[2].* 1) DDC Clinic for Special Needs Children, Middlefield, OH; 2) The Clinic for Special Children, Strasburg, PA.

DDC Clinic for Special Needs Children in Middlefield, OH is a community-based, primary care facility initiated 3 years ago for children with complex genetic and metabolic disorders, and is based on The Clinic for Special Children in Strasburg, PA. The philosophy of DDC Clinic embodies the concept of the medical home for children with special needs advocated by the American Academy of Pediatrics. The medical home is characterized as accessible, continuous, comprehensive, family centered, coordinated, compassionate, and culturally effective. DDC Clinic provides comprehensive care for over 200 patients, of whom 70% are Amish, and 30% are non-Amish. Many of the over 40 genetic and metabolic disorders seen at DDC Clinic are associated with founder genes, resulting in rare disease clusters in the community, such as prolidase deficiency, Cohen syndrome and ganglioside GM3 synthase deficiency. Patient diagnosis rate has increased from 22% to 67% over 3 years, which has dramatically enhanced disease management and family satisfaction. Although medical management for these patients remains challenging, effective care for some diseases, such as proprionic acidemia, can lead to nearly normal life. Only a small group of diseases are lethal, such as infantile cardiomyopathy and Amish nemaline myopathy, but palliative care for these patients is appreciated by the affected families. Patients also benefit from comprehensive medical care and care coordination. Through continuity of care, our increasing knowledge of each patient, and partnerships established with families, we have seen a significant shift from sick visits to preventive visits, and from high complexity visits to moderate complexity visits, from inpatient care to outpatient care, from emergent care to regular office visits, resulting in significantly decreased healthcare costs in this population. A recent anonymous survey revealed family satisfaction approaching 100%. We conclude that community-based medical home for patients with complex genetic disorders is not only medically care-effective but also cost-effective.

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Family history in rural primary care. *W. Feero, D. Meyer.* Dept Community/Family Med, Maine-Dartmouth Family Practice, Augusta, ME.

Purpose: To provide information on the state of family history data in the rural outpatient primary care setting. Methods: Survey completion by attendees to the 2005 annual state family practice academy meeting in a small rural state. The survey elucidated: 1)demographics, 2)attitudes towards the importance of family history, 3)the frequency with which family history data is obtained, and 4)preferences for the format of family history tools. Results: The 34 respondents included 23 primary care physicians, and 5 NP/PAs. Most were in full time practice, 80% saw greater than 50 patients per week. 70% had been in practice greater than 5 years; only 9% practiced in communities of 50,000 or more. 54% of providers had electronic medical records (EMR) in their practices; 96% had internet access in their practices with 37% reporting internet access in patient rooms. 79% reported that family history was frequently or almost always important to out-patient care. 100% frequently or almost always obtained some family history at patient visits. Only 18% reported creating a 3 generation pedigree frequently or almost always; 57% had never generated a pedigree. 89% reported never or very rarely using a formal family history tool; a single respondent used an internet-based family history tool. When asked to predict probability of their use of a family history tool developed using a paper, internet, or EMR format 64%, 7%, and 57% reported that they would be likely or very likely to use such a tool, respectively. Conclusions: In this sample, rural primary care providers recognized the importance family history and reported obtaining family history data at a high rate. However, adherence to formal guidelines for obtaining a family history was poor, about 60% had never generated a pedigree on a patient. The prevalence of internet and EMR technologies in these practices was high; however reported use and interest in using internet based tools for family history was very low. This data suggests that there may be significant barriers to the use of internet-based family history tools in primary care, and that paper-based family history tools or those integrated into an EMR environment might be more readily accepted.

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Copper Replacement Treatment for Symptomatic Menkes disease: Ethical Considerations. *P. Liu[1], S.R. Sheela[2], S.G. Kaler[1].* 1) Unit on Pediatric Genetics, Laboratory of Clinical Genomics, NICHD, NIH, Bethesda, MD; 2) Dept. of Pediatrics, Indira Gandhi Cooperative Hospital, Cochin, India.

Menkes disease is an often fatal X-linked recessive infantile neurological disorder caused by mutations in a copper-transporting ATPase (ATP7A). Subcutaneous injections of copper in Menkes disease restores circulating copper levels to normal. However, while early initiation of this treatment can lead to normal neurological development in affected infants, clinical outcomes in older, symptomatic Menkes patients have generally been disheartening. We address the ethical concerns surrounding whether copper treatment should be offered for Menkes disease infants diagnosed after neurological symptoms become manifest. These include: 1) the prospect for any benefits, 2) the potential risks and discomforts, 3) the parents wishes with respect to treatment, 4) the familys understanding of the treatments potential futility, 5) the familys understanding of the investigational nature of the treatment, 6) the potential for treatment to have an adverse impact on unaffected family members, 7) whether the ultimate decision regarding treatment should rest with health care providers or with the patients parents, and 8) the duration of treatment. An individual family report illustrates the problems and decision points encountered. We conclude that the ultimate decision on instituting a treatment with the possibility of small clinical benefits and improved patient comfort is best made by a patients parents. Clinical geneticists involved in the care of such families should provide full details on the limited benefits and potential risks of treatment, and offer adequate time for the parents to absorb and act on this information. The ethical issues encountered in providing investigative and possibly futile treatment for this difficult disorder seem relevant to other pediatric genetic conditions as well.

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The Formation of Advocates' Concerns about Policy Issues Related to Genetics and Genomics. *E. Alfano, J. O'Leary, J. Martin, R. Caligiuri, E. Terry, K. Christensen, R. van der Riet, N. Robinson, L. Wise, S.F. Terry.* Genetic Alliance, Washington, DC.

Purpose: To ascertain which policy issues disease advocates consider critical to the integration of genetic and genomic discoveries into medicine, and to determine what constitutes effective engagement of the advocacy community in these issues.

Methods: We surveyed 200 advocacy organizations, to determine which policy issues they considered critical to their organizational missions. We also analyzed the methods they used to make these determinations, particularly with regard to priority setting and resource allocation. We convened the advocacy community in a number of forums - meetings, listservs and teleconferences to discuss these issues and potential methods of impacting the policy debate.

Results: In general, the advocacy community measures the importance of policy issues in a pragmatic way, by determining the impact of the issue on translation of research to medicine. Resources are allocated by determining impact of the issue on the organization. Common policy topics included genetic nondiscrimination, reimbursement, access and disparities.

Conclusions: The voice of advocates and consumers is critical to the policy debate and may advance the integration of genomics into medicine. In addition, larger and more complex organizations integrate political considerations into health outcome concerns leading to a more complex policy priority setting scheme.

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Genetics websites: Quality, cultural content, and readability. *K.M. Kelly[1], R.B. Patrick[2], R. Shedlosky-Shoemaker[2], M. Katz[3].* 1) Human Cancer Genetics; 2) Dept of Psychology; 3) College of Public Health, Ohio State Univ, Columbus, OH.

The internet offers unprecedented opportunities for the communication of genetics information. However, the extent to which the internet will be useful to the general public will depend on the readability and quality of information conveyed. Studies of health-related websites have found that many have readability levels too advanced for the general population, which reads at or below the 8th grade level. The current study analyzed 55 genetics websites, identified through a National Institute of Health, Medline Plus internet search. Quality was analyzed consistent with guidelines from Health on the Net. Analysis of cultural content was guided by the Childrens Health Partnership. Readability Calculations software was used to compute 9 readability algorithms. In general, quality scores were high in ownership (98%), authoritativeness (98%), currency (96%), and interactivity (67%). Scores were lower in attributions (38%) and purpose (2%). Overall, little cultural content was included. Photos were uncommon (35%), but graphics were noted in most sites (82%). Although none of the sites were multi-lingual, some sites included links to multi-lingual (31%) and cultural (16%) sites. The average number of words per site was near 1,500. Reading levels were computed with the Flesch Reading Ease (M=38.9, SD=15.5), FOG Index (M=15.3, SD=3.1), Flesch-Kincaid Grade Level (M=12.5, SD=2.8), SMOG Tool (M=14.0, SD=2.1). Both the Flesch Reading Ease and FOG Index were more difficult than their target scores of 60 ($p<.001$) and 8 ($p<.001$). Both the Flesch-Kincaid Grade Level ($p<.001$) and SMOG Tool ($p<.001$) showed websites were written above the average grade level of the general population. Additional data regarding quality of genetics websites will be available at the time of presentation. In sum, the quality of websites identified through Medline Plus was high. However, most sites lacked cultural content and were written at a grade level too high for the general public. Efforts are needed to increase the accessibility of genetics websites to increase knowledge and awareness in the general public.

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Non genetics health professionals' confidence in caring for individuals and families with genetic conditions: Preliminary data from the European GenEd study. *I. Nippert[1], C. Julian-Reynier[2], H.J. Harris[3], L.P. Ten Kate[4], U. Kristoffersson[5], J. Schmidtke[6], C. Benjamin[7], K. Challen[7], R. Harris[7].* 1) Women's Health Research, UKM, Muenster, Germany; 2) INSERM, Marseille, France; 3) Brooklands Beacon Medical Practice, Manchester, UK; 4) University Hospital, Amsterdam, The Netherlands; 5) University Hospital, Lund, Sweden; 6) MHH, Hannover, Germany; 7) St Mary's Hospital, Manchester, UK.

Introduction: Non-genetics health professionals will increasingly be faced with individuals and families who have a need for genetics information and services. It is of increasing importance that non-genetic health professionals are ready to understand the role of genetics in health care and to provide adequate information and services. **Methodology:** In order to assess the preparedness to care for individuals and families with a need for genetic services, 12 genetic service items were listed in the GenEd questionnaire. The questionnaire was distributed in 5 European countries and more than 4.700 health professionals responded. Service items ranged from taking a family history to obtaining informed consent before taking blood for DNA-tests. Respondents were asked to report their confidence in their ability to carry out these services. **Results:** The majority of the respondents was confident or highly confident in identifying specialist genetic services in their area of practice (59%) or in taking a family history (55.9%), however, less than a third of the respondents were confident or highly confident in providing 8 out of 12 services. The lowest confidence rates were reported for counselling a woman for a predictive testing for Huntington's disease (9.5%), for recognising when malformations may be genetic in origin (12.7%) and for identifying patient support groups for rare genetic disorders (19.7%). Professionals with education in genetics were significantly more confident in providing 10 out of 12 services than professionals with no education in genetics. **Conclusion:** A coordinated international effort is needed among genetic professional associations and non-genetic professional associations to raise the level competence and confidence.

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Unpacking the Justifications for the Canadian Ban on Therapeutic Cloning. *C. Kurata, T. Bubela, T. Caulfield.* Health Law Inst, Univ Alberta, Edmonton, AB, Canada.

Introduction: In 2004, the Canadian Parliament enacted the *Assisted Human Reproduction Act*, using criminal law to ban all forms of human cloning, including somatic cell nuclear transfer (therapeutic cloning). The official justifications for this sweeping ban are stated in the Report of the Standing Committee on Health, *Assisted Human Reproduction: Building Families*, and in Health Canada media releases that accompanied a proposed version of the Act in 2001. These justifications are threefold: 1) concerns about the commodification of human reproductive capabilities and commitment to the ideal of human dignity, 2) concerns about public health and safety, and 3) claims of a social consensus respecting the impropriety of human cloning. Since it is Parliament - and not the bureaucratic machinery of Health Canada - which enacts Canadian law, discrepancies between the official justifications and those proffered in Parliamentary debate are both significant and worth documenting. This study examines whether such discrepancies exist.

Methods: First, we perform a qualitative, thematic analysis of the concerns about therapeutic cloning voiced in submissions to the Standing Committee on Health. Next, we see which of these submitted concerns resurface in the Committees Report. Finally, we analyze Parliamentary debates about the *Act* to see which justifications held sway at the moment of the legislations enactment.

Results: As the debate about the *Act* progressed from submissions to the Standing Committee to the final stages of Parliamentary debate, the tenor of the discussion shifted. From the plurality of arguments voiced in submissions, the three aforementioned official justifications emerged in the Standing Committees Report. Finally, Parliamentary debates - the final stage of political scrutiny - were marked by concerns about the moral status of the embryo.

Conclusion: The official justifications for the ban on therapeutic cloning do not reflect the concerns of the legislators who enacted it.

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Peter Severinus (1540-1602) and the Transplantation of Hereditary Disease. *M.T. Walton[1], R.M. Fineman[2].* 1) Independent Scholar, 16th Cent Studies Group, Salt Lake City; 2) Independent Scholar, Seattle.

The re-discovery of the works of Mendel and others has added greatly to our understanding of genetics. Such is now the case of Peter Severinus, with the recent re-discovery of his seminal work, Idea Medicinae Philosophicae (1571). Severinus concurred with Paracelsuss (1493-1541) concept of seeds (little chemical factories) that worked on matter to form living things; but he was also aware of transplantation (grafting and cross-pollination) that changed phenotypes and genotypes in plants. He applied this understanding to hereditary diseases in humans and extended Paracelsian theory. He believed that certain diseases in ones offspring were caused by a parents infected (abnormal) seed. Severinus also observed that some hereditary diseases skip generations, and some eventually lost their force and died out. Severinuss understanding of hereditary diseases also gave hope for their treatment, i.e., the chemical imbalance associated with a hereditary disease could be altered with the proper chemical remedy. Severinuss theory of hereditary disease was discussed and respected for almost a century but, eventually, it was forgotten as medicine moved towards a mechanical view of biological processes, and focused on the matter theory, measurement, and eventually Lavoisiers oxygen chemistry. Severinuss transplantation of hereditary disease was forgotten before the 19th century, although 19th century ideas of seed and germ plasm descend from Paracelsianism. His ideas were probably unknown to Mendel, who understood his own experiments in terms of the then current theory of germ plasm. Still, Severinuss budding understanding of hereditary disease shows that Paracelsian biochemical theory could yield a reasonable conceptualization of unseen processes, just as the theory of germ plasm did later. Severinuss coupling of keen observation with theory deserves to be re-discovered (a search of the U.S. National Library of Medicine web site finds little or no information about Severinus or his work) and acknowledged in much the same manner as Mendels work. Indeed, we can learn a great deal from how past thinkers conceptualized their theories and made observations.

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Comparison of Healthcare Professionals Responses to Ethical, Legal, and Social Issues in Cancer Genetics. *K. Banks, K.R. Blazer, J. Culver, K. Lowstuter, D.J. MacDonald, J.N. Weitzel.* City of Hope, Duarte, CA.

The evolving practice of genetic cancer risk assessment raises complex ethical, legal, and social implications (ELSI) concerning the appropriate treatment of genetic information. We surveyed health care professionals attending a one-day clinical cancer genetics education conference regarding their attitudes and knowledge about specific ELSI issues in clinical cancer genetics using hypothetical case scenarios. The 116 participants recorded their profession and responses (yes, no, or unsure plus explanation) to ten ELSI scenarios on an anonymous answer sheet which was collected prior to discussion of the scenarios. Reported professions included 32 doctors (MD), 23 nurses/student nurses (RN), 21 Genetic Counselors/genetic counseling students (GC), and 15 others. Most respondents recognized scenarios of potential genetic discrimination in health insurance (75%) and employment (91%), with no significant differences in knowledge between MDs, RNs, and GCs. For scenarios in which patient autonomy conflicted with duty to warn the patient, there were significant differences between professions, in that GCs favored patient autonomy, while RNs and MDs favored duty to warn ($P < 0.05$). Similar results were found in scenarios where patient confidentiality conflicted with duty to warn at-risk family members. Regarding the use of genetic testing to determine predispostion to an adult-onset hereditary cancer syndrome in a minor or for prenatal diagnosis, there were significant differences between professions with GCs being the least likely to facilitate testing in either scenario ($P < 0.001$). Differences in responses between professions likely reflect differences in their disciplines predominant approach to healthcare. Given the ethical challenges inherent in cancer genetic testing, involvement of a multidisciplinary team with complementary training backgrounds may be beneficial for the patient. Our findings suggest that while health care providers have basic knowledge about bioethical principles, the unique and dynamic nature of genetic information poses ethical challenges in practice which may be better understood through case-based training in ELSI issues.

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Is carrier screening for Gaucher disease type 1 justified? Lessons from the Israeli program. *S. Zuckerman[1,2], E. Levy-Lahad[1,2], A. Zimran[2,3], A. Shmueli[2], A. Lahad[2], M. Sagi[2,4].* 1) Medical Genetics, Shaare Zedek Medical Center (SZMC); 2) Hebrew University-Hadassah Medical School; 3) Gaucher Clinic, SZMC; 4) Medical Genetics, Hadassah University Hospital, Jerusalem, Israel.

Gaucher disease type 1(GD-1) is an autosomal recessive storage disorder. Presentation is variable, and includes hepatosplenomegaly, bone disease, and lack of neurological disease. One in 17 Ashkenazi Jews is a GD carrier. The most common mutation, N370S (75%), is mild, ~70% of homozygotes are virtually asymptomatic, and there is effective enzyme replacement therapy (ERT). Carrier screening for a low penetrant, treatable disease is controversial, but GD is part of the Ashkenazi panel and is widely offered in Israel since 1995. We conducted a nationwide study evaluating screening outcomes and experiences of at-risk couples identified. Between 1995-2003, ~35,000 persons had GD testing. 83 GD-1 carrier couples were identified, 65 (78%) of which were interviewed about decisions, knowledge, and attitudes towards GD/screening. 78% had prenatal diagnosis. Of 16 GD-1 fetuses diagnosed, pregnancies were terminated in 2/13(15%) N370S homozygotes and in 2/3(66%) N370S compound heterozygotes. Two of 4 couples who terminated pregnancies regret doing so. None of the GD-1 children (including older sibs) have required ERT (mean follow-up 5.6 yr). Attitudes toward pregnancy termination correlated with perceptions of GD as severe, of a high risk for severe disease in homozygotes, and with the expected genotype (with or without one severe GD allele). Most subjects recall getting minimal information pre-testing and only 55% felt post-testing counseling was sufficiently informative, though knowledge of GD was found to be very good. About 50% also met with a GD expert. 76% did not regret testing and 68% support program continuation. Despite high ERT costs, screening is not cost-effective since most couples do not terminate GD-1 fetuses. Though most couples perceived no harm, we conclude that GD-1 screening is unjustified: its benefits are unclear, it is not cost effective, and can result in questionable pregnancy terminations. If screening is performed, thorough genetic counseling is imperative to avoid harm.

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Recruitment to a familial cancer study: Balancing privacy protection and research quality. *J. Slutsman, B. Graubard, A. Garceau, G. Willis, L. Wideroff.* National Cancer Institute, Bethesda, MD.

Family-based research involving participation of index subjects and relatives is critical to cancer research. Little empirical data are available about factors associated with relatives participation in research, although this information is important for conducting studies. This research evaluates an investigator driven recruitment strategy enabling index subjects to first discuss the study with relatives. We assessed whether the index subjects willingness to give investigators permission to contact relatives was associated with their own demographic characteristics and other relative characteristics. In 2001, a random digit dial phone survey was conducted to determine the accuracy of family cancer history reported by 1,380 index subjects(response rate=70%). After obtaining a family roster and cancer history for 1st and 2nd degree relatives, a random sample of relatives was identified for validation of their cancer histories. Index subjects were given calling cards to facilitate discussion with relatives and were then asked to allow investigators to contact relatives. Determinants of contact permission were assessed using weighted multivariate logistic regression. Sixty-five percent of index subjects contacted at least one relative to discuss the study, although non-whites were less likely to do so than whites (OR=0.52 [0.32-0.84]). Furthermore, 67% gave investigators permission to contact some or all sampled relatives for an interview. After adjustment for educational level, index subjects were more likely to give permission to contact relatives with a history of cancer (OR=1.19 [1.08-3.37]), and those with whom they had a supportive relationship (OR=5.13 [2.66-9.92]). Overall, 90% of the 717 relatives whom the investigators were given permission to contact agreed to participate. With two-thirds of index subjects granting contact permission, this recruitment strategy was relatively successful, particularly among whites or for relatives with a supportive relationship or cancer history. This approach may enhance feasibility of population-based family studies and provide an effective balance between scientific objectives and ethical responsibility.

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Gender specific differences in the psychosocial adjustment of parents of a child with Duchenne Muscular Dystrophy (DMD): Two points of view for a shared experience. *A.A. Samson[1], S.A. Miles[2], M.C. Choquette[2], P.J. Jacob[1,2], P.K. Chakraborty[1,2], E.M. Tomiak[1,2].* 1) University of Ottawa, Ottawa, Ontario, Canada; 2) Children's Hospital of Eastern Ontario, Ottawa.

We have conducted a qualitative analysis of couples having a child living with DMD. Each member of the couple was interviewed separately. The focus of the semi-structured, open-questioned interview was on the key psychosocial aspects of the process of adjustment to the illness of their child (family and spousal relationships, daily life, emotions, career, spirituality, coping strategies). There was evidence of gender specific differences in constructing the different aspects of the shared experience. This finding has implications for the design and implementation of intervention strategies aimed at spouses caring for a child with DMD, or with other severe, chronic and uniformly fatal illnesses. Preliminary data of the in-depth interviews will be presented.

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Physicians' beliefs about the role of genetics in health differences and the importance of race/ethnicity and gender in clinical decision making. *E. Warshauer-Baker[1], V.L. Bonham[1], J. Jenkins[1], N. Stevens[2], Z. Page[1], A. Odunlami[1], C. McBride[1].* 1) Social and Behavioral Research Branch, National Human Genome Research Institute, Bethesda, MD; 2) University of Washington School of Medicine, Seattle, WA.

Recent research in genomics, pharmacology, and public health has sparked controversy over the use of race/ethnicity and gender in medicine. In particular, scholars argue whether the genetic contributions to group differences in health are overemphasized. There are little data on physicians' beliefs about these issues. We conducted a web-based survey of 1035 members of the American Academy of Family Physicians in January 2005, and we asked them the extent to which they thought racial/ethnic and gender differences in health were due to genetics and the environment. We also asked physicians to rate the importance of race/ethnicity, gender, age, family history, and insurance status in their clinical decision making. Overall, physicians surveyed reported that gender differences in health outcomes were due slightly more to genetics than to the environment (3.6 on a 7 point scale, where 1 = genetics, 4 = equally attributable, and 7 = environment; SD 1.3), and that racial/ethnic differences in health outcomes were due slightly more to the environment than genetics (4.6 on same scale; SD 1.2). In reference to their clinical decision-making, physicians rated age to be most important (5.9 on a 7 point scale, where 1 = not at all and 7 = essential; SD 1.1). Race/ethnicity (4.4; SD 1.4), gender (4.6; SD 1.5), family history (4.4; 1.5), and insurance status (4.3; SD 1.7) were rated similarly, and of less importance than age. Physicians' beliefs about racial/ethnic and gender health differences were not predictive of their ratings of the importance of race/ethnicity and gender in clinical decision making. To our knowledge, these data serve as a first, albeit modest, step toward understanding physicians' conceptualizations and conscious considerations of race/ethnicity and gender in their clinical decisions. Future research is needed to further elucidate these beliefs and better understand how they influence patient care.

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COST-BENEFIT ANALYSIS OF THE NEWBORN SCREENING PROGRAM OF THE PHILIPPINES. *C.D. Padilla[1,2], L.F. Dans[2,3], S.C. Estrada[1,2], M.R. Tamondong[1], R.M.S. Bernal[1].* 1) Institute of Human Genetics, National Institutes of Health Philippines, University of the Philippines College of Medicine, Manila, Philippines; 2) Department of Pediatrics, University of the Philippines-Philippine General Hospital; 3) Department of Clinical Epidemiology, University of the Philippines College of Medicine.

Objective: 1) To establish the incidence rate of CH, CAH, Gal, PKU and G6PD deficiency; 2) To determine whether the PNSP is cost-beneficial for each disorder individually or in combination from a societal perspective. Methods: The study was conducted through a screening survey of the 24 Metro Manila hospitals. Newborns were screened for CH, CAH, GAL, PKU and G6PD after the 24th hour of life. Newborns who screened positive underwent confirmatory testing. Using incidence rates from the survey, a population of 1.5 million and different screening combinations, the costs for the detection and treatment of the five disorders were compared to the benefits projected from preventing the corresponding complications and consequent productivity losses. Sensitivity analyses for varying incidence and discount rates were conducted to test the strength of the conclusions. Results: Of the 5 disorders, the most commonly detected is G6PD deficiency, followed by CH. Among the 5 disorders, only CH ($5.29 M) and G6PD deficiency ($15.44 M) had net benefits when screened individually. Pairing the disorders with CH showed benefit:cost ratios above 1, except for CH+PKU. Combinations of disorders also resulted in mostly cost-beneficial results, except when PKU was added. The total costs of screening of the 5 disorders amounted to $28.94 M, while the total benefits amounted to $40.36 M, yielding net benefits of $11.42 M. Conclusion: This study demonstrates that the benefits of a newborn screening program of the Philippines vs. a do-nothing alternative far outweighs the costs.

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Ethical Dilemmas in the Prenatal Diagnosis of Huntington Disease. *W. Hogge, M. Kolthoff.* Center for Medical Genetics, Magee Womens Hosp, Pittsburgh, PA.

Prenatal diagnosis is a well established reproductive technology. The principle goal of this technology is to diagnose fetuses with chromosomal abnormalities. Amniocentesis and chorionic villus sampling also may be used to diagnose classic genetic disorders of the fetus such as Tay-Sachs disease. Prenatal diagnosis for serious or fatal childhood diseases is generally well accepted. Current technologies also allow for the prenatal diagnosis of adult-onset genetic disorders such as Huntington disease (HD). It has been argued that prenatal diagnosis for HD followed by selective abortion is morally problematic because an affected fetus will likely experience decades of asymptomatic life. Furthermore, it is conceivable that some couples will decide to continue their pregnancy despite a prenatal diagnosis of HD. This scenario is worrisome because the future child may be harmed by their HD diagnosis while gaining little or no medical benefit from it. Finally, similar to the genetic testing of children, the prenatal diagnosis of HD in a continuing pregnancy raises concerns regarding future autonomy. The future child would not have a choice as to when or if to find out their true HD-status. Yet, in contrast, the PND of HD plays a vital role in the reproductive decision-making. Many at-risk couples feel strongly that they are responsible for preventing HD and its associated suffering in their children. Alternatives such as preimplantation genetic diagnosis with embryo selection or gamete donation are often prohibitively expensive, medically invasive, or do not allow for the preservation of genetic relationships between parent and child. Without PND, at-risk couples may be left to choose from natural conception, adoption, or to forgo child-bearing entirely. Limitation of reproductive autonomy to this degree requires demonstration of clear harm in order to be ethically reasonable. However, the proposed harms to future children have yet to be established or substantiated. Furthermore, the majority of pregnancies diagnosed with HD end in termination. In conclusion, the prenatal diagnosis of HD should be regarded as morally sound and should be available to at-risk couples in order to respect their reproductive autonomy.

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Navigating the ethical minefield between genetic research and clinical genetic services. *K. Hodgkinson[1], T. Young[1], S. Connors[1], A. Bassett[2], P. Parfrey[1], D. Pullman[1].* 1) Patient Research Ctr, Memorial University, St John's, NL, Canada; 2) Clinical Genetics Research Program, CAMH, Toronto, ON, Canada.

What responsibilities does a research team incur following the determination of clinically relevant genetic data for a serious disease with high recurrence risk and ameliorative interventions? Is the research team responsible for testing until clinical services can provide the results? Should research ethics boards (REB) require that provisions be made for clinical follow-up of subjects prior to project approval? Is it ethical to do genetic studies outside the locale of the primary research team unless there is local clinical follow-up of families? Should DNA results be seen as any other clinical test, and should a subject have the right to learn about a DNA result with health implications in the absence of permission from an affected relative? These questions have arisen in a Newfoundland research project on 16 families with autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC) that share a disease-associated haplotype at 3p25. This disease causes sudden cardiac death that can be treated effectively with implantable cardioverter defibrillators. DNA haplotyping within the context of a research project is part of the clinical work up. The lethality of ARVC has forced us to re-evaluate our procedures. Our consent form now requires that DNA results be given when available and we are considering a genetic Miranda statement to allow genetic information to be passed to family members if subjects cannot. Our molecular geneticists are covered for clinical liability by the University and Hospital. Our REB now considers the ability of local services to continue to follow and treat families after project completion as a criterion for project approval. Newfoundland has been the site of external research projects where no provision was made for clinical follow-up. These issues are doubly important in such circumstances as local clinical services must be able to respond effectively to increased demand. In recognition of such issues, draft legislation is in progress requiring that all Newfoundland research projects on human subjects be assessed by a provincial REB.

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The Use of Nude and Identifiable Photographs in Medical Genetics Textbooks: Ethical Considerations. *M.E. Burchett, A.L. Gustafson, C.A. Francomano, N.B. McDonnell.* Laboratory of Genetics, National Institute on Aging, Baltimore, MD.

Patient photographs are often used in medical genetics textbooks to illustrate phenotypes. Although such photographs are a valuable educational tool, utmost respect should be maintained for patient privacy and autonomy. Measures to protect the patients identity should be employed without compromising the educational value of the photographs. We encountered patients who have recognized their own nude photographs, taken during childhood, in medical textbooks and report that this is a distressing experience. We have reviewed sixteen major textbooks of medical genetics published in the last ten years for the presence of photographs of identifiable children and adults. Fourteen textbooks had at least one photograph of a completely identifiable nude patient. The range of such photographs was one to 217, and the mean number was 28. We located over one thousand photographs in which the patients full and identifiable face was depicted. The majority of nude and identifiable photographs represented minors. A statement on protection of patients rights to privacy, agreed upon by the International Committee of Medical Journal Editors in 1995, proposes that every measure should be employed to protect patient anonymity. The American College of Medical Genetics issued guidelines on informed consent for medical photography in 2000, stating that patients should be able to withdraw consent for the use of their photographs at a future date. The publication of an identifiable full face and/or nude photograph in a publicly accessible medical textbook makes the withdrawal of consent difficult to implement. We believe that the use of such photographs is not compatible with the spirit of protection of patient privacy, and should not have a role in medical textbooks. In the rare cases where a nude photograph is necessary to illustrate the genetic condition, the facial image should not be associated with the representation of the body. Efforts should be made to assure that the patient continues to give consent to the use of their images in subsequent editions of medical textbooks.

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Successful partners for community cancer genetic services: oncology practitioners and a nonprofit organization supporting individuals genetically predisposed to developing cancer. *A.S. Tranin[1], C. Nichols[2], S. Seidler[2], C. Boyne-Schuh[2], S. Ethirajan[1], A. Jew[3].* 1) Kansas City Cancer Centers, Overland Park, KS; 2) STAR (Supporting Those At Risk) Foundation, Overland Park, KS; 3) Breast Specialty, Menorah Medical Center, Overland Park, KS.

Cancer genetics is a rapidly evolving field, such that practitioners who do not specialize in the field are often not able to stay current with genetic discoveries and studies, or their psychosocial and ethical implications. The STAR (Supporting Those At Risk) Foundation is a unique nonprofit organization dedicated to the support of those who are genetically predisposed to developing any type of cancer. An oncology nurse specializing in genetics, a medical oncologist, a breast surgeon, three high-risk clients, and community leaders founded a successful high-risk support group in 1999 that evolved into the IRS designated non-profit organization, the STAR Foundation, in 2002. The founders recognized that many high-risk individuals were not appropriately identified, and their needs - emotional and medical - were often overlooked. In partnership with cancer genetic and oncology professionals, STAR promotes awareness of high-risk issues by presenting educational programs to healthcare providers and the public. STAR also provides access to cancer genetic counseling and testing for individuals who cannot otherwise afford these services. In addition, STAR directs a peer support group for those at risk for breast and ovarian cancer, assists in the placement of clients into suitable national clinical cancer genetics studies, and advocates for issues pertinent to high-risk individuals. STARs work assists a variety of community clinical practices in caring for individuals at risk. STAR has reached hundreds of diverse clients; helping to identify high-risk individuals and empowering them to manage their health proactively. The STAR Foundation is a successful example of an organization partnering with healthcare professionals to promote quality care, education, and advocacy designed for high-risk individuals.

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Report of an international survey of molecular genetic testing laboratories. *M.M. McGovern[1], R. Elles[2], E. Ronchi[3].* 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) National Genetics Reference Laboratory, Manchester, UK; 3) Organization of Economic Cooperation and Development, Paris, FR.

A detailed survey of molecular genetic testing (MGT) laboratories developed a group of international experts was conducted in 18 countries on 3 continents between June and October 2003(response rate 63%; n=827). QA and reporting indices were calculated for each responding laboratory to allow comparison of practices with other variables. Most (99.9%) directors had a doctoral degree, were certified to practice laboratory medicine (74%), and had training in molecular genetics (67%) although qualifications varied by country since some have specific legal and training requirements. Notably, the QA and reporting indices were significantly higher in laboratories that had directors with formal training in molecular genetics. Similarly, participation in proficiency testing (PT) was associated with a higher QA index which is important as it is known that PT programs are not currently available for many genetic tests, or are limited in their dissemination to certain countries or regions. Of interest, there was no statistically significant difference in the mean QA index between laboratories in different settings, or across the different countries, indicating that the QA standards included in the survey have penetrated widely. In contrast, there was a significant difference in the mean reporting index between research labs compared to all other settings ($p<0.05$). Since the majority of responding laboratories both receive (64%) and refer (60%) specimens across national boundaries, many of which are for rare disease testing carried out in research laboratories, this finding suggests that improvement in reporting practices is needed. The high rate of transborder flow of specimens also suggests the need to delineate best practices for informed consent, storage of specimens and confidentiality in order to promote confidence in genetic testing. The results of this survey which will be presented in detail document that MGT is provided under widely varying conditions and regulatory frameworks and may be a useful guide for policy action at both governmental and professional levels.

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A prospective study of reported genetic discrimination after screening for hemochromatosis and iron overload.

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Methods As part of the Hemochromatosis and Iron Overload (HH/IO) Screening (HEIRS) Study, 2037 participants from 6 states and Canada received genotype and phenotype screening for HH/IO and a subset received clinical examination. One year later, they reported whether they had any problems with insurance, employment, or blood donation that they attributed to HH/IO. 876 of respondents had HFE C282Y/C282Y or had elevated transferrin saturation and serum ferritin (TS/SF); 631 had lower indications of possible health risk such as H63D or other C282Y genotypes, or less serious TS/SF elevations; and 530 were control subjects with normal HFE genotypes and TS/SF levels.

Results 28 of 2037 HEIRS participants (1.4%) reported problems with employment or insurance they believed were related to HH/IO, and 56 (2.7%) reported being turned down as a blood donor due to HH/IO. 30% of problems were reported by participants who were diagnosed before the HEIRS Study. Among those not previously diagnosed who receive a clinical exam (n=792), 0.51% (4) reported a problem with employment, 0.76% (6) with health insurance, 0.76% (6) with disability insurance, 0.88% (7) with life insurance, and 3.79% (30) with blood donation. Among participants who were screened by not examined, the rate of reported problems was no greater in those with some indication of possible health risk than in controls.

Conclusions The incidence of reported genetic discrimination one year after screening for HH/IO is low, but is greater for those who underwent clinical examination, and is much greater for those who were previously diagnosed. This study was unable to determine whether problems increase over time or whether reports of discrimination were accurate.

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Organization, administration, and funding of state genetic services programs. *G. Wang[1], A. Roche[2], C. Watts[3], R. Carlson[3], D. Lochner-Doyle[2], Genetic Services Policy Project.* 1) Public Health Genetics, Univ. of Washington, Seattle, WA; 2) Genetic Services Section, WA State Dept of Health, Kent, WA; 3) Health Services Dept, Univ. of WA, Seattle, WA.

Purpose: The Genetic Services Policy Project describes the delivery of genetic services via state genetics programs. This review identifies policies and practices that may affect utilization of and access to genetic services. Given the dynamism of the health care system and subsequent effects on state budgets, evaluating state genetics programs gains importance as states critically examine their services. Methods: We systematically reviewed literature identified through PubMed and the GEMdatabase for information about the delivery of genetic services via state programs. Results: Analysis of the 18 states with genetics plans or needs assessments showed five categories of state genetics activities: 1) newborn screening; 2) testing and counseling services ranging from prenatal to adult diagnostic testing; 3) programs providing treatments to individuals with genetic disorders; 4) educational activities; and 5) birth defects surveillance programs. States vary in how they organize and fund care, education, and surveillance activities. Responsibility for these activities generally falls to either state genetics units or children with special health care needs units. Funding to support state genetics activities comes from service-related fees, state and federal governments, and private sources. Conclusion: States play an important role in delivering health services, including genetic services. Examining state systems and their effect on utilization of genetic services may reveal models for making access to services more equitable and administration more efficient. Changes in state-sponsored and publicly administered genetic service programs could substantially affect providers, payers, and consumers if public services are eliminated or transferred to the private sector. To continue its work in describing genetic services delivery models, the Genetic Services Policy Project plans to collect information from key stakeholders in order to translate genetics research into practice.

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Birth defects in Brazil: proposals for structuring and integrating care in the public health system. *D. Horovitz[1,2], J. Llerena Jr[1], R. Mattos[2].* 1) Instituto Fernandes Figueira, Rio de Janeiro, Brazil; 2) Universidade do Estado do Rio de Janeiro, Brazil.

The impact of birth defects (BD) in Brazil is increasing, shifting from fifth to second cause of infant mortality between 1980-2000, pointing to the need of specific health policy strategies. Actions related to BD in Brazil comprise teratogenic and metabolic disease information services, folic acid flour fortification, BD monitoring, newborn screening and treatment programs for some genetic diseases. Despite the importance of such initiatives, the organization of the genetic services as a functional network in the Brazilian public heath system (SUS) has not been contemplated. Aiming to map genetic services in Brazil, a questionnaire was sent all over the country, with data obtained from 60 services: 33 with clinical and laboratory services, 15 clinical, 8 laboratories and 4 medical teaching institutions. When estimating the need for clinical consultations related to BD, combining the research data and figures from the SUS database, there is clearly a consultation deficit. Regarding laboratorial support, an informal network operates among genetic services in the country, lacking official billing mechanisms. Propositions to improve care regarding BD are formulated, such as turning clinical genetics into an official medical specialty in the SUS, formalization of a national laboratory network with creation of billing mechanisms and the establishment of reference clinical and laboratorial centers integrated to the SUS. Also, a national BD registry based on birth certificates and educational programs for medical and general population are advised. It is presently unlikely in the SUS, where most decisions regarding health care are based on county administrations, that BD can be viewed from an integrated standpoint. A specific national policy regarding BD must be formulated, contemplating general care and alternative funding and billing options. Only with the active participation of the Ministry of Health, using the existing genetic services as a dorsal spine, will it be possible to structure a regionalized, hierarchical and functional network for the care of BD in Brazil.

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Licensure of genetic counselors: lessons learned in Oklahoma. *A.E. Weedn, S.J. Hassed, J.J. Mulvihill.* Section of Genetics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Licensure for genetic counselors is imperative to provide accountability and quality assurance to patients receiving genetic counseling. One state, Utah, has implemented licensing, California and Illinois have achieved legislation, and several others are at various points in the legislative process. The Policy Committee of the Oklahoma Genetics Advisory Council (*Am J Hum Genet* 2000;69:A62) initiated efforts to create legislation to license the states genetic counselors. The Genetic Counseling Licensure Act, modeled after the Utah and California bills, was drafted and circulated to the leaders at NSGC, ACMG, and ASHG to solicit feedback. The bill defined the profession and scope of genetic counseling, described licensure and continuing education requirements, prohibited other healthcare professionals from representing themselves as genetic counselors, and directed the State Board of Health to promulgate rules and licensure requirements. The bill unanimously passed the Health Committee in the Senate, but was challenged on the Senate Floor due to concern regarding the potential for abortion counseling. An amendment modifying the scope of practice was adopted, and the bill passed the Senate Floor. In the House Health Committee, the bill met opposition from the Chair of Oklahomans for Life, sparking a debate regarding the bills presumed potential for increasing abortions in the state. Ultimately, the bill (available at www.lsb.state.ok.us/) failed to pass out of committee by a narrow vote despite support voiced by a majority of non-committee Representatives. The bill will be reintroduced next year with plans to focus on quality assurance and access to care. Lessons learned from this experience include: the need for effective lobbying at every step of the legislative process with early formation of a lobbying coalition, simplifying language, partnering with national and local organizations to gain support, distributing compelling documentation on the importance of licensure, and educating the public and legislature on the practice of genetic counseling and the need for setting a professional standard of quality care.

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Releasing Results of Genetic Testing to Research Participants: A Multidisciplinary Consensus Statement. *M. Cho, S. Tobin, H. Greely, D. Magnus, J. Illes, CIRGE Genetics Research Policy Working Group.* Ctr Biomedical Ethics, Stanford Univ, Palo Alto, CA.

With recent advances in molecular technologies, a wide variety of genetic research projects now involve large-scale genotyping of individual participants. For example, such studies may seek to define the genetic contribution to disease, or may look for correlations between sequence variations and pharmaceutical safety or effectiveness, or may assess large populations to detect interactions between environmental exposures and heritable susceptibilities. All investigators engaged in the design and conduct of such projects are faced with the dilemma of whether, how, and when to report findings from genotyping to those who participate in their research studies. These findings may be genetic testing results specifically collected as part of the study, or DNA sequence of clinical significance but incidental to the purpose of the study. A dizzying array of medical, social, legal, and ethical factors can be considered, each of which may influence study design, conduct of informed consent, dissemination, composition of research teams, and availability of resources. Most principal investigators, proposal reviewers, and research administrators are not prepared to address these complex issues and their implementation.

In the summer of 2005, the Center for Integration of Research on Genetics and Ethics (CIRGE) at Stanford University invited a group of nationally known genetics researchers and ethicists to attend a workshop to address these issues. A consensus among these experts was sought through interactive case-based exercises, targeted presentations, small group discussions, and deliberations involving all attendees, with a charge to craft detailed recommendations useful for researchers, policymakers, research administrators, and funding agencies. The goal of this group effort is to create a flexible tool that will assist with creation and evaluation of projects that advance knowledge in genetics and acknowledge the complexity of handling genetic information about individuals appropriately.

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Bringing Science and the Law Together. *M.W. Sharp[1], J.P. Evans[2], T. Caulfield[1].* 1) Health Law Inst, Univ Alberta, Edmonton, AB, Canada; 2) Departments of Genetics and Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Introduction: Science and the law have traditionally been perceived as distinct cultures. The knowledge, training, and philosophies of scientists are divergent from those of lawyers and judges. In modern practice, the increased interaction between science and law is demonstrated, for example, by the use of genetic information as forensic evidence and by government regulation of genetic research. Science and law can no longer act as completely separate pursuits, and an understanding of their differences is necessary to achieve a synthesis to confront important emerging societal issues. This study will examine how science and the law interact and will highlight the differences between the two cultures. **Methods:** An analysis of pertinent case law, legislation, and professional organization policy statements was performed to examine the roles of science and the law and how the two disciplines interact. **Results:** The scientific and legal cultures serve different roles in society. While the ultimate goal of uncovering the truth is arguably common to both positions, there are different tools available and limits upon each to pursue this goal. While science can generally be described as an active pursuit of answering questions, the law has a passive role, addressing conflicts as they arise. In modern North American society, science is subservient to the law, allowing scientific pursuits to be restricted and regulated by legislation and court decisions. **Conclusion:** This examination of science and the law demonstrates that the fundamental differences between scientists and members of the legal profession are a potential source of confusion and conflict between the two cultures. Further analysis of these differences can lead to a better understanding of scientific-legal interactions, the development of an interaction model, and an enhanced method of confronting important societal issues.

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Public Consultation About Reproductive Genetic Testing: An Evaluation of Two Methods. *J. Scott, K. Hudson.* Genetics & Public Policy Ctr., Johns Hopkins University, Washington, DC.

Public consultation is used to understand the publics attitudes about a specific issue and the values that shape those attitudes. A public consultation process known as deliberative democracy brings together experts, policymakers and fellow citizens to learn from each other and deliberate about the issue. It requires broad and representative participation, balanced and accurate information, and an opportunity to deliberate with fellow participants. Unlike other countries, there have been few public consultation activities in the U.S., especially on the topic of human genetics. We evaluated two deliberative democracy models on reproductive genetic testing. METHODS: *The Genetic Town Hall: Making Every Voice Count* was held in six cities using a modified town hall format and separately conducted online with 15 discussion groups. Informational videos were used to ensure uniform content in each setting. The town hall participants were recruited using community outreach. The 3-hour, scripted sessions provided a balance of pre-session polling, education, facilitated discussions, and post-session polling. Online participants, recruited from a representative web-enabled panel, took an 80 item online survey, met online for one-hour moderated discussions weekly for three weeks, then took a 76 item post-discussion survey. A randomly selected control group completed identical pre- and post-surveys. RESULTS: 536 participated in the town halls; 133 online. Significant shifts in attitudes occurred in both groups, most notably about appropriate uses of reproductive genetic testing and the need for oversight. The town halls attracted more knowledgeable stakeholders; online participants were more representative of the general public. Town halls generated media coverage and the involvement of community leaders; online groups had more detailed discussion but had limited wider impact. CONCLUSION: As genetic discoveries move from research into practice, public consultations such as these can provide a mechanism for public education and dialogue with experts and policymakers. Depending on the goals of the public consultation, however, each method has benefits and drawbacks.

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Community Engagement and Genetic Variation. *S.F. Terry[1], P. Johnson-Moore[2], K.P. Deignan[2], H. Martinez[3], S. Metosky[4], T. Citron[4], Public Engagement in Genetic Variation and Haplotype Mapping Community Advisory Committee.* 1) President and CEO, Genetic Alliance, Washington, DC; 2) Iona College, New Rochelle, NY; 3) Westchester County Department of Health, Westchester, NY; 4) University of Michigan, Ann Arbor, MI.

This project assessed public reaction to the complex concepts of genetic variation, haplotype mapping and disease susceptibility in distinct populations. Using the suburb of a major city as the engagement and educational research site, this project invited community organizations, their members and the general public to participate in focus groups, public dialogue activities and a conference designed to explain, explore and examine these new scientific concepts.

The research team acquired background knowledge, established community oversight and advisory boards, convened diverse focus groups to develop an initial list of genetics issues, developed educational and issue-oriented materials relating to the issues list, convened community-based dialogue groups to select issues of concern and carry out a series of dialogue sessions, held a one-day Community Conference and disseminated the results back to the community and to other interested parties.

The communities concluded that these issues needed to be communicated to scientists working on genetic variation: disparities and access, privacy, control/decision making, identity, nonmedical uses, anthropological issues and definitions of health and disease.

This project was funded by the Ethical, Legal and Social Implications of National Human Genome Research Institute (NHGRI) through the Communities of Color and Genetics Policy Project at the University of Michigan. [NIH-ELSI GRANT: # 2 RO1 HG01005-01].

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Islamic Ethical Guidelines in Genomics and Genetics: A public Health Initiative. *A. Al-Aqeel*[1,2]. 1) Dept Pediatrics, Riyadh Military Hosp, Riyadh, Riyadh, Saudi Arabia; 2) Dep of Genetics, King Faisal Specialist Hospital and Research centre.

We are at a time of unprecedented increase in knowledge of rapidly changing technology. Such biotechnology especially when it involves human subjects raises complex ethical, legal, social and religious issues. A WHO expert consultation concluded that genetics advances will only be acceptable if their application is carried out ethically, with due regard to autonomy, justice, education and the beliefs and resources of each nation and community. Public health has a poor positioning in the health system, with lack of importance and support, poor dialogue with the publics and under skilled public forces, with lack of importance and support to, and long gap between intervention and outcome. Public health authorities are increasingly concerned by the high rate of births with genetic disorders especially in developing countries where Muslims are a majority. Therefore it is imperative to scrutinize the available methods of prevention and management of genetic disorders. A minimum level of cultural awareness is a necessary prerequisite for the delivery of care that is culturally sensitive, especially in Islamic countries. Islam presents a complete moral, ethical, and medical framework, it is a religion which encompasses the secular with the spiritual, the mundane with the celestial and hence forms the basis of the ethical, moral and even juridical attitudes and laws towards any problem or situation. Islamic teachings carry a great deal of instructions for health promotion and disease prevention including hereditary and genetic disorders, therefore we will discuss how these teachings play an important role in the diagnostic, management and preventive measures including: genomic research; population genetic screening (premarital screening, pre-implantation genetic diagnosis; assisted reproduction technology); stem cell therapy; genetic counselling and others.

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Beyond technomedicine: the human aspects of Preimplantation Genetic Diagnosis. *C. Bouffard.* Pediatrics, Université de Sherbrooke, CHUS, Sherbrooke, Québec, Canada.

A recent multisite ethnographic research project in medical anthropology, conducted in France at PGD-accredited centres in Strasbourg, Paris and Montpellier, on the representations that researchers, physicians and patients entertain with respect to Preimplantation Genetic Diagnosis, showed that couples who opt for the technique entertain substantially different representations than those put forth by the bioethics milieux. This is particularly so of concepts regarding eugenism, the perfect child, the embryo, biological filiation, and the selfishness and narcicism that are associated to such concepts, as well as the abuse/misuse of reproductive biotechnology and its relatively limited therapeutic use, and patient vulnerability.

Such representations go beyond the technomedical approach. As such, PGD is perceived not only as an act of love toward ones partner and unborn child, but also as an expiatory rite, a means of assuming responsibility and compensating for the biological incapacity to avoid that child the affliction of serious disease. The ritual of PGD affords such couples the opportunity to demonstrate courage, love, a desire to conform, and a spirit of sacrifice, each of which also empowers both partners.

Our data suggest that PGD patients have developed novel representations with respect to reproduction and ethical concepts which coherently integrate the individual, familial, cultural, social, moral, technological and medical levels, and enable the perception of PGD as a human practise that offers informed couples the means to protect their offspring from an invalidating genetic combination. This makes it all the more difficult to elaborate guidelines and bioethical rules, with respect to reproductive genetics, without knowing or taking into account the points of view and experiences of patients, despite the fact that their representations, of what may be ethical or not, differ from those of the experts that preside the elaboration of such norms.

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Taking a Family History from a Cultural Perspective. *C. Fernandez[1], F. McKain[1], I. Harlow[2], N. Robinson[3], S.F. Terry[3], P. Kyler[4], M. Puryear[4], J. Boughman[5].* 1) Institue for Cultural Partnerships, Harrisburg, PA; 2) American FolklifeCenter, Library of Congress, Washington, DC; 3) Genetic Alliance, Washington, DC; 4) Genetic Services Branch,MCHB,HRSA, Rockville, MD; 5) ASHG, Bethesda, MD.

Healthy Choices through Family History Awareness is a project developed by the Institute for Cultural Partnerships in collaboration with the American Folklife Center, the American Society of Human Genetics and the Genetic Alliance through a cooperative agreement with the Genetic Services Branch of Maternal and Child Health Bureau. Using an approach designed to tap into a familys own oral history and traditions, the folklorists and colleagues have developed a consumer-based family history tool that encourages and provides a format for individuals and families to organize and preserve family history and health related records. The Allison Hill area of Harrisburg, Pennsylvania, with a high density of low income African American and Latino populations, is marked by significantly poor health outcomes including diabetes, heart disease and stroke, cancer, and infant mortality, all conditions in which genetic factors play a critical role. Methods in tool development have included focus group feedback, field testing, and training of community advocates to encourage and assist families. By using this tool, we seek to increase genetic literacy among African American and Latino individuals and families residing in the area. The project has identified family practices regarding genetic health information management. Coupled with consumer education, the project will enable family members to better communicate about potential genetic risk factors among themselves and with their health care and social service providers. The project will serve as a model for increasing awareness of family history and its relevance to health for both consumers and providers.

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Towards a New Paradigm: Redefining Race, Ethnicity & Underserved Communities in Light of Genetic Advances. *L. Wise[1], N.T. Robinson[1], R. van der Riet[1], G.C. Christopher[2], P. Kyler[3], M. Lloyd-Puryear[3].* 1) The Genetic Alliance, Washington, DC 20008; 2) Health Policy Institute, The Joint Center for Political & Economic Studies, Washington, DC 20005; 3) Genetic Service Branch, Maternal & Child Health Bureau, HRSA, HHS.

Purpose: In the light of recent advances sequencing & interpreting the human genome, some posit that human race does not meaningfully exist at the genetic level, while others think it does. The impact & validity of this debate is being studied by many organizations, including the National Commission on Health, Genomics & Human Variation.

Methods: A report is being prepared on how the field should interpret & respond to this debate. Through substantive research, including focus groups, the Genetic Alliance has developed a new paradigm on the roles that race, ethnicity & underserved communities should play in field of health care that considers current genetic resources & the future of health in America. The Genetic Alliance has developed a broad dissemination plan, including training sessions & workshops, to encourage the consideration and use of this new paradigm in health care.

Results: The production of a teachable framework & workshop for a new philosophy & practice of considering race, ethnicity & underserved communities in health care will be delivered to the field.

Conclusions: In order to reduce health disparities & absorb genetic resources, new ways of thought & practice in health care regarding race, ethnicity & underserved communities are required.
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Gene expression profiling distinguishes subsets of patients with Sjogrens syndrome and sicca symptoms. *E.S. Emamian, L. Tobon, J. Leon, A.N. Leiran, C.M. Meyer, E.C. Baechler, T.W. Behrens, A. Huang, B. Segal, N.L. Rhodus, K.L. Moser.* University of Minnesota, Minneapolis, MN.

Sjogrens syndrome (SS) is a complex autoimmune disease defined primarily by clinical manifestations related to dry eyes and dry mouth (sicca symptoms). Initial studies in our lab using microarray technology revealed over expression of Interferon (IFN) inducible genes in peripheral blood of 34 SS patients when compared with 22 normal subjects. All 34 patients fulfilled the 2002 revised European criteria for SS and 91% were seropositive for anti-Ro/SSA and/or anti-La/SSB autoantibodies. Overexpression of the IFN inducible gene cluster was also positively correlated to titers of anti-Ro and anti-La. In the present study, we have evaluated 14 additional sicca patients who were clinically diagnosed with SS based on subjective symptoms of both dry eyes and dry mouth, but were seronegative and failed to meet the 2002 criteria due to either unavailable or inconclusive labial gland histopathology. Comparing gene expression in peripheral blood of Sicca vs. normal subjects identified 1,043 differentially expressed transcripts ($p<0.001$ and average fold change of >1.5). Comparison of SS patients vs. normals identified 246 transcripts, 30 of which overlapped with genes identified in the Sicca group. Hierarchical clustering was used to visualize patterns of differentially expressed genes across all patients (n=56) vs. controls. The majority of SS patients clustered together. However, 13/14 of the Sicca patients clustered together and displayed a gene expression pattern that was clearly distinct from the SS patients with a prominent IFN signature. Genes that best distinguish SS patients include IFI17, IFI15, IFIT3, OASL, TNFAIP6 and IRF7. In the Sicca group, unique genes include EEF1D, RAPGEF6, MAP3K3, TNFSF14, and transportin 1. Genes that are differentially expressed across all patients include HLA-G, STAT1, IFNAR2, TNFSF10/TRAIL and IgG heavy chain. These results indicate that subsets of patients with sicca manifestations can be identified using microarrays and that different pathways may be involved in the pathophysiology of patients with sicca symptoms.

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Identification of some common pathways among autism spectrum disorders. *N. Kaya, M.S. Inan, M. Asyali, N. Sakati, M. Nester, P. Ozand.* Genetics, KFSH&RC, Riyadh, Saudi Arabia.

Autism is a neuropsychiatric, neurobehavioral disorder with a wide spectrum of diseases. In most situations, autism is the result of abnormal function of multiple genes or a few genes with pleiotropic effects, and in many cases the inheritance is affected by imprinting. We hypothesize that the individual disorders existing with the autism spectrum disorders might share common disturbed physiological and molecular pathways. For this purpose we have selected three disorders with different autistic phenotypes but all of which manifest autism; Rett syndrome, FragileX with autism, and Early and severe infantile autism. We performed microarray analysis of gene expression using Affymetrix HG-U133 Plus 2.0 in order to determine the gene signatures by detecting the altered molecular pathways. We will present several altered pathways that share common denominators.

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Analysis of Mouse Inner Ear MPSS Libraries to Select for Rare and Novel Expressed Genes. *L.M. Peters, I.A. Belyantseva, J.F. Battey, T.B. Friedman, R.J. Morell.* National Institute on Deafness and Other Communication Disorders, National Institutes of Health (NIDCD/NIH), Rockville, MD 20850, USA.

Massively Parallel Signature Sequencing (MPSS) is a powerful technique that allows in-depth gene expression profiling by generating millions of 20-base signatures from the 3 end of mRNA samples. We have developed MPSS libraries of mRNA isolated from dissections of postnatal day seven (P7) mouse organ of Corti, stria vascularis and vestibular sensory epithelium tissues (libraries named MoCR, MoSV and MoVB, respectively). There are a combined total of 29,672 signatures in the three libraries, after removing non-reliable or non-significant signatures. Of these, 7,848 are unique to the inner ear, by comparison with other available MPSS libraries from a variety of mouse tissues. The most abundant of these signatures identify the 3 ends of genes already well known to be prevalent in the inner ear. For example, signatures for Otogelin are the most abundant unique signatures in the MoVB library, while signatures for Otospiralin are the most abundant unique signatures in the MoCR library. The low abundance signatures are especially interesting, since they should derive from rare transcripts present at 10 to 15 copies per cell. This should equate to approximately 40 times per million (tpm) for a signature from a low abundance message in a homogeneous tissue. Since the abundance levels can be estimated to as low as 4 times per million (tpm), these libraries should reveal signatures even for rare mRNAs expressed in specific cell types in the complex tissues of the inner ear. We have identified signatures to rare genes unique to the inner ear and have confirmed the expression and structure of these genes by RT-PCR and in situ hybridization.

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Downstream molecular analysis of gDNA eluted from Formalin-Fixed, Paraffin Embedded Tissue (FFPET) using a modified GenSolve Method, stored on GenVault GenPlates, and recovered using GenSolve. *J.A. McEvoy, G. Veber, J.R. Tierney, D.T. Wong, M.E. Hogan, J.C. Davis.* BioScience, GenVault Corporation, Carlsbad, CA.

The aim of this study is to evaluate the quality and purity of genomic DNA isolated and stored from formalin-fixed, paraffin embedded tissue (FFPET) using a new method compared to a organic solvent based method. Genomic DNA from normal and malignant FFPE breast, liver and kidney tissue was isolated using two methods: GenVault Recovery Solution plus proteinase and a organic solvent based extraction. Fifteen microliters of the recovered DNA slurry was aliquoted onto each GenPlate element and dried prior to room temperature storage. Using the GenSolve DNA Recovery Kit the gDNA was isolated from the element to test for quality and purity. PCR amplification, sequencing, genotyping, SNP analysis, and whole genome amplification was preformed on gDNA isolated using the GenVault Method and organic solvent based methods. Results indicate the quality and purity of the gDNA isolated using both methods were comparable. The GenVault protocol does not require toxic solvents or temperatures above 60 C and provides sufficient sample for long-term, room temperature storage on GenVault GenPlates. Downstream molecular analysis can be performed using GenVaults gDNA recovery method from FFPET samples stored on GenPlates. The system and product is compatible with all GenVault semi-automated and automated storage and retrieval systems.

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Comparative Genomic Characterization of Neuro-Episodic Disease Candidate Genes. *J. Freudenberg, Y.-H. Fu, L.J. Ptacek.* Laboratories of Neurogenetics, UCSF - Department of Neurology, San Francisco, CA.

Neuro-episodic diseases are known to share important characteristics both on the molecular level and the phenotype level. In the present study, we systematically identified protein sequence families and functional categories, which are associated with neuro-episodic disease genes. As expected, the most significant results were obtained for proteins with synaptic localization or for proteins involved in synaptic transmission. A relatively high number of disease genes with synaptic function is also reflected by their significant enrichment among the full set of human disease genes. We therefore hypothesized that genes with synaptic function posses an increased potential to harbor more penetrant disease mutations. If true, this could be reflected in an increased evolutionary conservation of those genes. Therefore, we next analyzed phylogenetic profiles of neuro-episodic disease genes and disease candidate genes with known synaptic function. We found both groups displayed an increased number of orthologs in non-primate vertebrate genomes. When examining sequence evolution of human-rodent ortholog pairs, we observed increased overall constraint on neuro-episodic disease genes as well as on synaptic candidate genes. Thus the enrichment of genes with synaptic function among human disease genes is correlated with their retainment in the genomes of other species and with a decreased sequence evolution. The genetic complexity of neuro-episodic disease phenotypes, therefore, could at least partially result from an increased likelihood of deleterious mutations in genes playing a role in synaptic function.

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The Analysis of Array-CGH Data: A Change-point Approach. *N.R. Zhang, D.O. Siegmund.* Statistics Department, Stanford University, Stanford, CA.

Array-based comparative genomic hybridization (array-CGH) measures the chromosome copy number at multiple loci simultaneously, and is useful for finding the regions of genome deletion and amplification in tumor cells. However, array-CGH data is noisy, with the signals corresponding to chromosomal aberrations hard to detect. We model array-CGH data as a Gaussian change-point process, and propose a new statistic for determining the number of chromosomal aberrations. We call our statistic the "modified BIC" because of its similarity to the Bayes Information Criterion, which is a classic statistic for model selection but not applicable to this change-point problem.

We tested our method on both the simulation data from Olshen et al. (2003) and the array-CGH data sets from Snijders et al. (2001) and Snijders et al. (2003). The results show that the modified BIC is preferable to existing methods for statistical analysis of array-CGH data. The modified BIC has the advantages of being prior independent and impartial, and also much simpler to use than most of the current procedures. The method can also be applied to other problems involving model selection of change-point like problems.

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Comparative analysis of chimpanzee full-length cDNAs mapped onto human genome unveils potentially large inter-species divergence in gene loci. *R. Sakate[1,2], Y. Suto[3], T. Imanishi[2], I. Hayasaka[4], T. Gojobori[2,5], K. Hashimoto[6], M. Hirai[7].* 1) Integrated Database Group, JBIRC, JBIC, Tokyo, Japan; 2) Integrated Database Group, BIRC, AIST, Tokyo, Japan; 3) 2nd Research Division, Japanese Red Cross Central Blood Institute, Tokyo, Japan; 4) Kumamoto Research Park, Sanwa Kagaku Kenkyusho CO.,Ltd., Kumamoto, Japan; 5) Center for Information Biology and DNA Data Bank of Japan (DDBJ), National Institute of Genetics, Shizuoka, Japan; 6) Division of Genetic Resources, National Institute of Infectious Diseases, Tokyo, Japan; 7) International Center for Molecular, Cellular and Immunological Research, Tokyo Women's Medical University, Tokyo, Japan.

Chimpanzees are our closest relatives in the evolutionary lineage and their genome sequences are highly similar to ours. In spite of the genetic similarity, there exists distinctive divergence between humans and chimpanzees, particularly in cognitive abilities and morphological traits. In order to elucidate the cause of the divergence at the transcript level, we constructed chimpanzee full-length cDNA (FLcDNA) libraries. In this study, chimpanzee cDNA sequences representing 87 gene transcripts were analyzed with the mapping information onto human genome. We found that, by comparing the alignable sequence pairs, the degree of diversity, including substitution and insertion/deletion (indel) events, in 5'-untranslated region (UTR) and 3'-UTR was smaller than that of synonymous substitution in coding sequence. We also found that the transcription start site (5'-UTR end) was more variable than the transcription termination site (3'-UTR end), and that putative species-specific alternative splicing occurred more frequently in 5'-UTR while large indels occurred more frequently in 3'-UTR. These results imply that different types of functional constraint exist between 5'-UTR and 3'-UTR along the human and chimpanzee lineages. Finally, given all the small and large-scale sequence divergences considered, the chimpanzee transcripts differed from their human orthologous counterparts by 2.6% in nucleotide sequences, which was larger than the difference formerly estimated.

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Highlights of Genetic and Genomic Resources at NCBI. *D. Maglott, NCBI staff.* Natl Ctr Biotechnology Info, National Library Medicine, NIH, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) continues to increase the depth and breadth of the many databases and services that are accessed by millions of users daily. Building on the foundation of publications (PubMed, PubMedCentral, Books), sequence (GenBank, RefSeq) and maps (CancerChromosomes, Map Viewer), NCBI provides resources focused on genes (dbMHC, Gene, HomoloGene, UniGene), protein sequences and structures (TaxPlot, COGs, CDD, COGs, BLink), expression (GEO, GENSAT), phenotypes (GeneTests, OMIM, OMIA, PhenoDB), small molecules (PubChem), and variation (dbSNP). Recent developments of note include several new Entrez databases (GENSAT, Genome Project, OMIA, PhenoDB, Probe), tools to align mRNAs to genomic sequence (Splign), a robust client to analyze and annotate sequences (Genome WorkBench), enhancements to BLAST, and improved coverage and interrogation of such model organisms as bee, cow, dog, chicken, opossum, sea urchin, and zebrafish. More alternate assemblies of key genomes have been made available to complement official ones. The focus of the presentation will be the use of Genome Workbench and NCBI's databases in the areas of probe identification, gene structure, comparative genomics, and correlating sequence and phenotype.

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Predictions and analyses of mutable sequences flanking the repeats in Friedreich's ataxia and Fragile X syndrome. *V. Handa, B.E. Wright.* Division of Biological Sciences, University of Montana, Missoula, MT-59812.

Genetic instabilities of triplet repeat sequences including $(CTG)_n(CAG)_n$, $(CGG)_n(CCG)_n$ and $(GAA)_n(TTC)_n$ have been found to associate with at least 14 genetic neurodegenerative diseases. Evidence suggests that DNA secondary structures formed by abnormally long repeats block the progress of DNA polymerase, resulting in extended pause sites and further expansions (or contraction) of these repeats during slippage replication. We have developed a computer algorithm *mfg* that can predict the location of unpaired, mutable bases in the secondary stem-loop structures [SLS(s)] of single stranded DNA during transcription. For a chosen window size, or length of nucleotides, *mfg* folds that number of nucleotides successively, beginning with each base in the sequence analyzed. The output of the program shows the percent of all folds in which each base is unpaired and the most stable (-G) SLS(s) in which it is unpaired and therefore most likely to mutate. We have used *mfg* to examine sequences flanking the repeats in two trinucleotide repeat disorders [Friedreichs ataxia (FRDA) and Fragile X syndrome (FXS)] with *mfg*. The program predicted intrinsic mutability of the sequences flanking the repeats. In the region upstream of the GAATTC repeats in FRDA, 21 mutable bases (2 deletions and 19 base substitutions) have been identified (Bidichandani et al., 1999).Different bases mutated in normal individuals compared to symptomatic individuals. In fact, *mfg* found different populations of SLS(s) in these normal individuals compared to symptomatic individuals and it could be shown that most of the mutable bases in symptomatic individuals were rarely unpaired while in normal individuals most of the mutated bases were unpaired. Similar analyses by the *mfg* program of the sequences flanking CGGCCG repeats in FXS have predicted that about 33 nucleotides 5 and 58 nucleotides 3 of the repeats are mutable. To verify the *mfg* predictions, we are amplifying these regions in normal, premutated and mutated Fragile X cell lines by polymerase chain reaction and sequencing them.

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SNPselector: a web tool for selecting SNPs in genetic association studies. *H. Xu, E.R. Hauser, M.A. Hauser, S. Züchner, S.G. Gregory, J.E. Stenger, M.A. Pericak-Vance, J.M. Vance.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Single nucleotide polymorphisms (SNPs) are the most common form of polymorphism in human genome. They are commonly used in genetic association studies of complex diseases. With the recent advance of SNP genotyping technology, researchers are able to assay thousands of SNPs in a single experiment. However, the process of manually choosing thousands of genotyping SNPs is time consuming. We have developed a web program, SNPselector, to automate this process. SNPselector is implemented in PERL language. It can be run as a UNIX command-line application, with a CGI wrapper to provide the web interface. A local MySQL database is developed as back end of SNPselector to store SNP information, including SNP location, allele and allele frequency, function, validation, and HapMap genotyping data. To increase the program performance, Ensembl gene structure and UCSC genome annotation data are downloaded and stored in the local database as well. The local database is updated whenever new public data are released. SNPselector takes a list of gene names or genome regions as input and finds all available SNPs in the genes or genome regions. SNPs with experimentally-verified genotyping or allele frequency information are considered as high quality SNPs. SNPselector finds tagging SNPs by calculating LD bins of genotyped SNPs. It then finds SNP function based on whether the SNP may affect the gene transcript structure or the protein product. It checks the regulatory potential of the SNP based on SNP location at conserved site from multi-genome comparison, conserved TFBS site, CpG island, or miRNA gene. It also checks whether the SNP is in a repeat region. Finally it prioritizes SNPs on LD tagging property, SNP quality, function, regulatory potential, and repeat status. The SNP selection result is put into Excel files. Users can do further analysis on the SNP result files to select SNPs with specific features. Output formats include selected SNPs along with flanking sequences to Illumina or other companies for SNP assay design. Its code and database files are available upon request.

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easyLINKAGE - Automated linkage analyses using microsatellite or large-scale SNP data. *K. Hoffmann[1], T.H. Lindner[2].* 1) Dept Medical Gen, Humboldt Univ, Charite, Berlin, Germany; 2) Department of Nephrology, Clinical Biochemistry/Pathobiochemistry, Medical University Clinic at the University of Würzburg, Würzburg, Germany.

Most of linkage analyses software was traditionally developed for UNIX environments restricting calculations to a limited number of experienced users. The time-consuming generations of input files as well as missing or very basic graphical outputs are further limitations. Nowadays, scientists prefer to perform their own analyses right after generating the genotypes. However, an automated tool for performing linkage analyses under the same operating environment where scientists get their daily PC work done was not available so far. We have developed the program easyLINKAGE that covers all those issues. It combines automated setup and performance of linkage analyses and/or simulation studies under an easy to handle graphical user interface for Microsoft Windows 2000/XP. The current version of the program supports FastLink, SuperLink, SPLink, GeneHunter/-PLUS/-Imprinting/-TwoLocus, Allegro, Merlin, SimWalk, and FastSLink with automatic loop breaking. Further, we integrated conditional multipoint analyses. easyLINKAGE provides genome-wide and chromosomal plots of LOD scores, NPL scores, P values, and many other parameters. The program generates input files for pedigree/haplotype drawing software such as HaploPainter. The program can test for HWE deviations, NON-/Mendelian inheritance problems. Data can be analyzed for predefined chromosomal intervals and in marker sets. The latter option enables the handling of thousands of markers on a chromosome. easyLINKAGE can simulate multipoint linkage data and performs subsequent linkage analyses according to the users model. To run the program marker files containing subject IDs/genotypes, a pedigree structure file (LINKAGE format), and a marker database are required. All options for the covered linkage programs can be set interactively. easyLINKAGE boosts the use of most known linkage programs under a Windows environment and enables to perform analyses for a wide audience (http://www.uni-wuerzburg.de/nephrologie/molecular_genetics/download.htm).

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Modeling of Dynamic Genetic Networks. *Y. Sun[1], L. Jin[3], M. Xiong[2].* 1) Statisitcs, Fudan University, Shanghai, Shnaghai, China; 2) Human Genetics, University of Texas Health Scince Center at Houston; 3) School of Life Scicne, Fudan University, Shanghai, China.

A complex biological system has two states: equilibrium and disequilibrium. Static models of genetic networks are unable to reveal important information on how a biological process changes from one state to another or how to respond to environmental stimuli. It is also widely recognized that the regulatory interactions between genes in the networks can be fully revealed only through perturbation of biological systems. Therefore, a systematic understanding of these processes requires the development of mathematic tools for modeling dynamic genetic networks. Although dynamic Bayesian networks are emerging as a promising tool for dynamic genetic network modeling, they do have limitations for genetic network identification and analysis. First, dynamic Bayesian networks cannot model feedback structure of networks. Second, it is very difficult to develop nonlinear dynamic Bayesian networks. In this report, we propose to use dynamic structural equations as a basic tool for modeling dynamic genetic networks, which allow to model feedback structure of the networks and can incorporate dynamic Bayesian networks as a special case of dynamic structural equations into the dynamic genetic network modeling. Three-stage least square methods will be used to estimate the parameters in the networks and genetic algorithms will be employed to identify the structure of the genetic network which best fit the data. The statistics for testing the interactions between the genes in the networks will be discovered. The proposed dynamic structure equation models will be applied to cell cycle and drug response analyses.

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Design of multiplexed oligionucleotide ligation assays for high throughput insertion/deletion and single-nucleotide polymorphism genotyping. *R. Koehler, Z. Zhang, N. Peyret, J. Day, S. Short, M. Wenz, F. De La Vega.*
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Insertion and deletions are important mediators of disease and disease susceptibility. We developed a multiplexed high throughput assay based on oligonucleotide ligation to determine genotypes of insertion/deletions and SNPs. The assay platform is based on the oligonucleotide ligation assay (OLA) followed by universal PCR and utilizing capillary electrophoresis as the readout. Three oligonucleotide probes address each SNP or insertion/deletion (indel) polymorphic loci with sequences including target site-specific and universal components. Target site-specific portions are designed to meet thermodynamic and specificity requirements, ensuring that probes bind efficiently and exclusively to only the intended polymorphic target sites. Universal components must not interfere with probe binding and probe sets must function without interfering with other probes in a multiplex. Potential interferences include probe-probe interaction, probes acting as ligation templates for other probes, and probes that inadvertently ligate to probes designed for another target loci. Avoiding regions of low sequence complexity and secondary structure also improves assay robustness. These rules are implemented in an automated design pipeline. We now describe design improvements aimed at increasing conversion rate, assay performance, specificity, and multiplex level for both SNPs and indels. Heuristics are used to screen candidate probes and design steps are ordered to reduce intensive calculations. For evaluating genomic uniqueness, we developed a fast and sensitive genome screening algorithm that takes the OLA reaction explicitly into account and uses empirically-based match/mismatch weights around each polymorphic loci. Our multiplexing algorithm uses information from the above screens together with specific probe-probe checks in a swapping strategy that partitions assays into mutually compatible multiplexes. The result is a set of OLA probes that target addressable polymorphisms and are mutually compatible within a multiplex. Comparable success rates of SNP and indel genotyping are demonstrated.

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An ensemble stepwise regression approach to gene mining. *D.Y. Yang[1], X. Li[1,2], S.Q. Rao[1,3], J. Li[1].* 1) Department of Bioinformatics, Harbin Medical University, Harbin 150086, China; 2) Department of Computer Science, Harbin Institute of Technology, Harbin 150001, China; 3) Departments of Cardiovascular Medicine and Molecular Cardiology, Cleveland, OH 44195, USA.

Gene mining based on genome-wide transcriptional profiling of complex diseases has been increasingly recognized for identification of disease genes. In this study, we present an ensemble-based stepwise regression-support vector machines approach (termed ensemble SR-SVM) for hunting for the feature genes for complex diseases. The ensemble SR-SVM, a significant extension of the tree-based ensemble approach proposed previously by us, was developed to exploit the relative merits of the two core computational algorithms for analysis of high dimension microarray data. We conducted a numerical experiment to explore the ensemble SR-SVM using a public dataset of colon cancer for both identification of disease relevant genes and biological classification. Compared with several established gene mining methods, the ensemble SR-SVM achieved better performance on extracting the feature genes in terms of higher disease relevancy values, and also yielded better classification accuracy for predicting colon cancer samples.

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Tuning GENEHUNTER for personal computers. *S. Bolay, E. Bagnoud, D.F. Schorderet.* IRO - Institute of Research in Ophthalmology, Sion, Switzerland.

Purpose: GENEHUNTER (gh) is a linkage software developed by Kruglyak et al (1996). Based on parametric and non-parametric algorithms, it has helped identifying many loci and associations and has become, one of the most popular linkage tool freely available to the scientific community. Unfortunately, the full capabilities of gh can only be appreciated when running on fast computers. Typically, its use on personal computers may increase the computing time up to several hours in complicated families. We therefore reengineered the source code in order to accelerate the execution time.

Methods: The reengineering of the source code was performed on a Linux operating system (processor Intel PIV 3GHz, 1GB of RAM). First, the code was tested to measure the time used for typical applications. Then, it was analyzed in order to identify and quantify the time used by each discrete function. We identified the top 10 functions in time consumption and concentrated our efforts in reengineering them by moving calls to variables outside of loops (while, for,...) and avoiding calls to variables which were pointers to pointers. The reengineered gh version was compared using real pedigrees from our linkage and mapping activity.

Results: The time needed by gh to perform calculations on our machine was decreased from 3420 to 1845 by changing the compilation optimization mode from level 0 to 3. Moreover, improving the source code in the top 10 functions has decreased the calculation time down to 501.

Conclusion: A simple re-writing of the most frequently used functions improved the speed of gh by a factor of 7. In-depth reengineering should even increase the speed of the program further .

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A Flexible Framework for Data Mining and Knowledge Discovery in Human Genetics. *J.H. Moore[1], J.C. Gilbert[1], C.-T. Tsai[2], F.-T. Chiang[2], W. Holden[1], N. Barney[1], B.C. White[1].* 1) Computational Genetics Laboratory, Department of Genetics, Dartmouth Medical School, Lebanon, NH; 2) Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Detecting epistasis or gene-gene interactions in studies of human disease is a computational and a statistical challenge. To address this problem, we have previously developed a multifactor dimensionality reduction (MDR) method for collapsing high-dimensional genetic data into a single dimension (i.e. constructive induction) to facilitate statistical modeling of interactions. Here, we describe a comprehensive and flexible framework for detecting and interpreting epistasis that utilizes 1) advances in information theory for selecting interesting single-nucleotide polymorphisms (SNPs), 2) MDR for constructive induction, 3) machine learning methods for classification, and 4) graphical models for interpretation. We illustrate the usefulness of this strategy using artificial datasets simulated from several different two-locus and three-locus epistasis models. We show that the accuracy, sensitivity, specificity, and precision of a naïve Bayes classifier are significantly improved ($P < 0.05$) when SNPs are selected based on their information gain (i.e. class entropy removed) and reduced to a single predictor using MDR. We then apply this strategy to detecting, characterizing, and interpreting epistatic models in a genetic study (n=500) of atrial fibrillation and show that both classification and model interpretation are significantly improved ($P < 0.05$). All methods and procedures are implemented using open-source and freely-available software packages. The MDR approach is written in JAVA and is freely available from www.epistasis.org as part of the open-source Weka-CG software package. Inclusion of MDR in Weka-CG makes it possible to integrate this powerful method for modeling interactions with a wide variety of existing machine learning tools for data transformation and normalization, variable selection, classification, and visualization. Combining MDR with variable selection procedures will make it possible to detect epistasis in genome-wide association studies.

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Design and validation of resequencing primer sets for 16,336 human genes for analyses of sequence variation and SNP discovery. *X. Lin[1], J. Ni[1], Z. Zhang[1], G. Merkulov[1], J. Hoover[1], M. Rydland[1], R. Sanders[1], P. Ma[2], C. Heiner[2], A. Lakdawalla[2], M. Martinyak[2], P. Li[1], E. Spier[2].* 1) Applied Biosystems, 45 West Gude Dr. Rockville, MD 20850; 2) 850 Lincoln Centre Dr. Foster City, CA 94404.

Increasing attention has been focused upon SNP discovery and genotyping in an effort to associate disease/phenotypes with gene variations and mutations, and to determine evolutionary relationships, but reliable primer design and data analysis remain two of the major challenges.

high-throughput, high quality computational pipeline has been developed to design PCR primer pairs for resequencing of human genes. Primer design is driven by the primer3 program and supplementary algorithms developed at the Applied Biosystems. All known SNP/MNP sites in the genomic sequence are avoided during primer design. The resulting primers are then QAed by two computational tools: the first one checks the primer pairs for genomic redundancy using a proprietary version of the e-PCR program with refinements for mismatch adjustment; the second tool, developed using a wet-lab validated training set of 200K amplicons, then predicts the probability of success of the primer pairs. Project Template files containing information for the gene targets are generated to allow integrated analysis workflow with the Seqscape software.

We are in the process of designing resequencing sets for all 16336 human genes with Refseq sequences. Annotation data from 20,617 RefSeqs and from 27,499 Celera transcripts have been combined to define the structure of these genes. For the 5176 genes for which design has been completed, we have achieved coverage of 92% of all bases (coding exons, UTRs and 1kb upstream) and 94% within the coding regions. On average 21 amplicons are required to sequence a whole gene and 13 to sequence just the coding region. For validation, 800 randomly selected amplicons were lab-tested, with an overall success rate of 95%.

Complete designs and associated data will be released to the research community.

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SNP Selection Algorithm for Optimal Density and Information Content. *B.G. Kermani, A. Oliphant.*
Bioinformatics, Illumina, Inc, San Diego, CA.

The recent utility of multiplexed SNP genotyping arrays emphasizes the importance of the selection of a set of appropriate and informative SNPs. A selection process is required as the number of available SNPs identified by the SNP Consortium (~9 million) considerably outnumbers the number of SNPs one could place on the current high-end multiplexed genotyping arrays (~500,000). Even upon completion of the HapMap Project, SNP selection will remain crucial, as the number of expected Tag SNPs may be larger than the capacity of the state-of-the-art genotyping arrays. There are two main parameters in SNP selection: distribution in the region(s) being studied, and the informativeness of the set. Informativeness can be estimated using various quality metrics of specific SNPs, such as validation level, uniqueness, proximity to repetitive regions, and minor-allele frequency. Our algorithms utilize an iterative process that starts with an empty set or a set of user-defined mandatory SNPs. At each iteration, one SNP is added to the set, based on the relative performance metric of a SNP to the final score. A SNP is selected that contributes most to the final score. This score is composed of the static and dynamic components. The static score is fixed for each SNP throughout the iterative process, and includes several parts, e.g., MAF, validation and uniqueness scores. The dynamic score changes at each iteration, and is based on the landscape made by the set of mandatory and already selected SNPs, using a Parzen estimator model with Gaussian neurons. At each iteration and for each of the candidate SNPs, the dynamic score is computed and subsequently combined with the corresponding static score via a Sugeno fuzzy inference system. The algorithm can be adapted to use the haplotype block boundary information. In the simplest form, the spread of each neuron could be set to the corresponding length of the haplotype block for a certain Tag SNP. This algorithm has been successfully used to select SNPs for HapMap, linkage and fine map panel applications.

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Exploring structural roles of evolutionarily conserved residues in two-dimensional contact map space. *Z. Li*[1,2], *U.K. Muppirala*[2]. 1) Department of Chemistry & Biochemistry, University of the Sciences in Philadelphia, Philadelphia, PA; 2) Bioinformatics Program, University of the Sciences in Philadelphia, Philadelphia, PA.

Protein structure prediction remains a fundamental challenge in the post-genomics era and understanding protein sequence-structure relationships is essential to the development of computational tools for structure prediction. Evolutionarily conserved amino acid residues are generally assumed to play a structural or functional role in proteins. Understanding how they contribute to the integrity of a proteins three-dimensional structure individually is valuable to protein structure prediction. We propose to study the contributions of conserved residues via contact maps derived from their structures and then examining the pattern of connectivity. We systematically applied this method to diverse fold-type sets of proteins. Several distinguishing features of conserved and conserved hydrophobic residues were identified: (i) Within a native fold, the average degree of connectivity increased in the order of all residues < conserved residues < conserved hydrophobic residues; (ii) The percentage of residues occupying the high end of the distribution of degree dimension followed the same order of all residues < conserved residues < conserved hydrophobic residues; (iii) The range of the maximum degree of connectivity of any residue in a native fold was between three and eight; (iv) The majority of residues with the maximum degree of connectivity are either completely or highly conserved hydrophobic amino acids. We give a test case in which we applied these rules to help differentiate native from non-native structure.

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A Web-Based Java Application for Genotype and Phenotype Database Management. *J.-L. Li[1,2], M.-X. Li[2], H. Deng[3], H.-W. Deng[2,3,4].* 1) Seattle Biomedical Research Institue, Seattle, WA; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China; 3) Osteoporosis Research Center, Creighton University Medical Center, Omaha, NE; 4) The Key Laboratory of Biomedical Information Engineering of Ministry of Education, and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P.R.China.

In genetics and genomics studies for complex human diseases, it is not unusual that large numbers of genotype and phenotype data points are acquired. Efficient and effective management of a vast amount of genotype and phenotype data is then necessary. Previous software developed in MS ACCESS for large-scale management of genotype data has its limitations, i.e., Operating System (OS) dependency, limited database capacity and lacks of functions for management of phenotype data. To overcome these limitations, we developed a web-based Java application for genotype and phenotype database management (GPDB). GPDB has been developed based on Java 2 Platform, Enterprise Edition (J2EE). One advantage of Java programming is OS independency. As a result, GPDB can virtually run on any major Operating Systems including Windows, Linux, and UNIX. In addition, instead of MS ACCESS, MySQL has been employed as the backend database server. Comparing to MS ACCESS, MySQL can handle larger amount of data sets gracefully; therefore, the database capability has been increased dramatically. Moreover, new functions to manage phenotype data have been developed and incorporated into GPDB. Entity-Attribute-Value (EAV) model has been applied to develop the phenotype data management system. By applying the EAV model, this application allows users to manage arbitrary phenotypes and customize data entry forms; therefore it is suitable for different and multi-center projects. Last, phenotype data, pedigree data and genotype data can be compiled together in GPDB for further data analysis or directly feeding linkage analysis software such as QTDT and GENEHUNTER. The test version of GPDB is publicly accessible at http://apps.sbri.org/gpdb.

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Now you see it, now you dont. *T.P. Sneddon[1,2], E.A. Bruford[1,2], T.A. Eyre[1,2], V.K. Khodiyar[1,2], R.C. Lovering[1,2], M.J. Lush[1,2], K.M.B. Sneddon[1,2], C.C. Talbot Jr[1,3], M.W. Wright[1,2], S. Povey[1,2].* 1) HUGO Gene Nomenclature Committee (HGNC); 2) Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London, NW1 2HE, UK; 3) The Johns Hopkins School of Medicine, Institute of Genetic Medicine, The Johns Hopkins University, Baltimore, MD, 21205-2196, USA.

The HGNC has to date approved over 21,000 unique symbols and names, the majority of which are for genes, i.e. genomic segments that are transcribed and translated into functional proteins.

What happens when these genes, initially thought to be single copy in the human genome, turn out to be variable in copy number between individuals? This is the case for an increasing number of genes with already approved symbols, including members of the well-established amylase, defensin, protocadherin and blood group antigen gene families.

It has yet to be determined to what extent these additional gene copies are functional, what effect they have on genome stability and disease susceptibility, and how they contribute to individual phenotypic differences. Whatever their impact they will inevitably need to be discussed in the literature, preferably using a meaningful and systematic nomenclature that is globally accepted.

So it falls to the HGNC to meet the needs of the scientific community by addressing this issue. Examples of variable copy number genes and the potential problems they pose to the current nomenclature system will be presented alongside proposals for additional/alternative nomenclature schemes. We would welcome your suggestions and comments so please visit us at our booth in the Exhibit Area, email us at nome@galton.ucl.ac.uk, or go to http://www.gene.ucl.ac.uk/nomenclature for further information.

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A new portal as an aid for mutation collection. *C. Talbot Jr[1], R.G.H. Cotton[2, 3].* 1) Inst Genetic Medicine, Johns Hopkins Sch Medicine, Baltimore, MD; 2) Genomic Disorders Research Centre Fitzroy, VIC 3065, Australia; 3) The University of Melbourne Parkville, VIC 3010, Australia.

Single gene disorders affect around 1% of all births and mutations have been estimated to affect 60% of all humans in their lifetime. Despite this there has been no systematic attempt to collect all mutations. Some collection has occurred in general databases but it has been limited due to funding or commercial restraints. This collection has, by necessity, been centralised without the involvement of experts in genes.

In 1994, an initiative began to attempt to form a federation of curators of lists of mutations in genes (locus specific databases or LSDBs) to attempt to collect and curate all mutations in all genes in an expert manner. Since that time, problems to be faced have been solved and procedures have been put in place to such an extent that in 2000 all that was required was funding. Funding has been elusive but considerable progress has been made (see this and earlier progress on www.hgvs.org).

The ultimate aim is for the clinician/diagnostician to have instantly at their finger tips, all reports of mutations and their effects in a complete and accurate manner in a standard format. Progress towards this end includes: 1. Availability of new software from those wishing; to curate mutations in their gene of interest (http://www.dmd.nl/LOVD/1.1.0/); a portal, 2. A plan for a dedicated central database, 3. Numerous recommendations and publications which will be detailed in the presentation and 4. The Waystation, through which mutations can be channelled to a journal for publication and a Pub Med ID, currently undergoing beta testing (http://www.centralmutations.org/).

A group of 20 scientists have been identified so far testing submissions and a sample submission will be presented. This submission is based on a submission form developed by 38 members of the Human Genome Variation Society.

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Expansion of GAA trinucleotide repeats in mammals. *R. Clark, S. Bhaskar, M. Miyahara, G. Dalgliesh, S. Bidichandani.* Dept Biochem & Molec Biol, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK.

We have previously shown that GAA trinucleotide repeats have undergone significant expansion in the human genome. Here we present the analysis of the length distribution of all ten non-redundant trinucleotide repeat motifs in 20 complete eukaryotic genomes (six mammalian, two non-mammalian vertebrates, four arthropods, four fungi and one each of nematode, amoebozoa, alveolate and plant), which showed that the relative expandability of GAA trinucleotide repeats is specific to mammals. Analysis of human-chimpanzee-gorilla homologs revealed that loci with large expansions are species-specific and have occurred after divergence from the common ancestor. PCR analysis of human controls revealed large expansions at multiple human (GAA)30+ loci; nine loci showed expanded alleles containing >65 triplets, analogous to disease-causing expansions in Friedreich ataxia, including two that are in introns of genes of unknown function. The abundance of long GAA trinucleotide repeat tracts in mammalian genomes represents a significant mutation potential and source of inter-individual variability.

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Customizations To GeneMapper For Use In a High Throughput Genotyping Environment. *B. Craig[1], B. James[2], S. Newman[2], M. Barnhart[1], C. Boyce[1], K. Hetrick[1], J. Romm[1], C. Bark[1], Y. Tsai[1], E. Pugh[1], C. Boehm[1], K. Doheny[1].* 1) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Applied Biosystems Informatics North American Professional Services, Foster City, CA.

The Applied Biosystems (AB) GeneMapper software was designed to replace and add to the functionality of previous Genescan and Genotyper fragment analysis programs. GeneMapper software allows for simultaneous sizing and genotyping of capillary electrophoresis data from Applied Biosystems 3730 and 3700 genetic analyzer platforms. GeneMapper software did perform sizing and genotyping of data with minimal user review in addition to creating or modifying binned allelic data; however many limitations to the program made it difficult for use in a high throughput genotyping environment. CIDR contracted with Applied Biosystems NA Professional Services to modify the released GeneMapper software in order to accommodate interaction with CIDRs new LIMS and increase its functionality for use in a high throughput genotyping lab. Enhancements to the GeneMapper software graphic user interface involved modifications to the size match editor, adding defined review comments to an individual or group of genotypes from a list of user-defined values, an analysis option for re-analyzing samples by marker(s), a modified panel manager plot viewer to quickly check outlier data points and ability to view all florescent dyes in the allele size range of a selected genotype. In addition to program modifications new features were developed to allow for integration of the GeneMapper database schema and aspects of the 3730 Unified Data Collection software into CIDRs new master database structure. Additions include real time inter-run size adjustments, a server to local database project Check-out application, autonomous serialization and migration of the 3730 run data into CIDRs central database and data integrity checks based on CIDR QA/QC standards. Overall, we expect the customized GeneMapper software product will allow CIDR to increase throughput per technician, reduce the need for manual review and increase the speed of data analysis.

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1,000 KAO-NASHI (Face-less) genes and their functional annotation. *A. Shimizu[1], S. Asakawa[1], T. Sasaki[1], M. Furutani-Seiki[2], H. Kondoh[2], N. Shimizu[1].* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Kondoh Research Group, SORST, JST, Japan.

The Human Genome Project has provided the DNA sequences of 3 billion base pairs and identified over 23,000 protein-coding genes in the human genome. However, many of these protein-coding genes are not fully proven by experimental evidences. In general, proteins with known motifs are readily classified, but substantial numbers of protein have no obvious motifs in their sequences. We designated these genes/proteins without obvious motifs as KAO-NASHI (Face-less) and initiated a project to unveil their face (kao) by comparative genomics and knockdown analysis using Medaka (a small teleost fish) as animal model. We extracted 1,000 KAO-NASHI genes from whole human genome using motif analysis by InterPro, homology search by BLAST and document search by PubMed. In order to unveil these KAO-NASHI genes, we decided to use Medaka (Oryzias latipes) as a model animal because it is easy to obtain embryos and to observe the organs or anatomical structure. We have identified Medaka orthologs for over 60% of human KAO-NASHI genes using the Medaka EST and whole genome shotgun sequence databases. A series of cDNA extracted from Medaka embryo was used for the first annotation of KAO-NASHI genes. Developing stage of Medaka embryo was divided into 39 stages based on the diagnostic features of the developing embryos. We extracted mRNA separately from each stage and used for expression analysis of KAO-NASHI genes. Many of the initial 100 genes showed ubiquitous expression during development. However, several genes showed gastrula, neurula or organs forming stage-specific expression. These expression profiles are useful for initial functional annotation. The gene knockdown strategy provides information on gene function by showing the effects of a drastic reduction in expression of the target gene. Morpholino oligonucleotides (MOs) injection has been successfully used to knockdown gene expression in Medaka embryo. We will present some morphological changes during Medaka development due to knockdown of several KAO-NASHI genes.

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PrimerTile: designing overlapping PCR primers for resequencing. *P. Chines, A. Swift, L.L. Bonnycastle, M.R. Erdos, J. Mullikin, F.S. Collins, NIH Intramural Sequencing Center.* NHGRI, NIH, Bethesda, MD.

PCR-based resequencing is currently the optimum method to exhaustively identify all of the variants that exist in a given genomic region among a set of DNA samples. Existing software can adequately design PCR primers in coding regions and other non-repetitive DNA. However, a comprehensive search for functional variants should also include as much as possible of the large fraction of genomic sequence that shows homology to known repeats. Successful amplification of these regions is more difficult.

We have created software, called PrimerTile, to optimize primer selection in resequencing projects. PrimerTile uses several techniques to avoid non-specific amplification, including several different repeat masking strategies and low-stringency electronic PCR, taking advantage of the strengths of each. In order to achieve the greatest accuracy, most SNP-discovery programs require complete reads from both strands. High quality double-stranded reads cannot be achieved when long homopolymer (or certain microsatellite) repeats are included in PCR products, since slippage of the polymerase leads to varying numbers of bases in the repeats, resulting in out-of-phase traces and poor sequence quality downstream of these regions. PrimerTile avoids including homopolymers in products, instead anchoring primers in or near such repeats, minimizing the size of the regions that cannot be analyzed.

PrimerTile efficiently covers the target region with PCR fragments of the specified size, and with as few gaps as possible. The program also avoids placing primers on top of known variants, in order to maximize the success of PCR amplification and sequencing. We report our experience with using this program, including the proportion of sequence captured by the designed PCR amplicons, the rate of successful amplification, and the proportion of amplified bases resulting in high quality bi-directional sequence.

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Automation of the Affymetrix GeneChip Mapping 100K Set. *H. Hobbs[1], C. Bark[1], M. Barnhart[1], R. King[1], E. Kwasnik[1], J. Strand[1], R. Mei[2], M. McKown[3], J. Cahlik[4], K. Doheny[1].* 1) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore MD; 2) Affymetrix, Santa Clara, CA; 3) PerkinElmer (PE), Boston, MA; 4) Acme Automation, Spring City, TN.

Standard protocols for Affymetrix GeneChip Mapping 100K Set involve manual processing of small sample sets. CIDR requires laboratory and informatics automation for high throughput environments in order to reduce human error. Many labs running the 100K set, or other Affymetrix platforms are processing samples in a single tube format. CIDR is leveraging its knowledge base and resources to process samples in a 96 well plate format. CIDR collaborated with Affymetrix to incorporate unique positive controls that validate correct sample position for each experiment and detect cross contamination. Robotic automation programs were developed using the Beckman Biomek 2000 and FX for the Digestion and PCR steps. PEs Multiprobe II was utilized for PCR plate consolidation, sample addition to the hybridization master mix, and chip injection which is informatically validated to ensure correct sample/chip association. Access to the chips was made possible via collaboration with PerkinElmer and Acme Automation. On the informatics side, the Affymetrix sample experiment importer was extended to allow for batch uploads of sample experiment combinations, including chip barcode, into the GCOS software, a time consuming process that could lead to incorrect data being entered manually. The end result is a process that utilizes robotics for the majority of the sample handling steps involved, incorporating various methods of QA/QC to ensure the data does not become compromised.

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Repetitive sequence environment distinguishes housekeeping genes. *C. Eller[1], M. Regelson[1], B. Merriman[1], S. Nelson[1], S. Horvath[1,2], Y. Marahrens[1].* 1) UCLA Department of Human Genetics David Geffen School of Medicine, Gonda Center, 695 E. Young Drive South, Los Angeles, California 90095-7088, USA; 2) UCLA Department of Biostatistics, School of Public Health, Gonda Center, 695 E. Young Drive South, Los Angeles, California 90095-7088, USA.

Housekeeping genes are expressed at high levels and across a wide variety of tissues. In view of reports of repetitive sequences influencing gene expression, we investigated whether an association exists between the breadth and magnitude of housekeeping gene expression and their repetitive sequence context. We show that Alu elements are concentrated around housekeeping genes while several other repetitive sequences, including LINE-1 elements, are excluded from these regions. These properties, in combination with other previously published sequence properties of housekeeping genes, were used to develop a method of predicting housekeeping genes on the basis of DNA sequence alone. Using expression across tissue types as a measure of success, we demonstrate that repetitive sequence environment is by far the most important sequence feature identified to date for distinguishing housekeeping genes. We also examined whether relationships exist between repetitive sequence environment and the breadth and magnitude of expression throughout the entire genome. We find that Alu abundance correlates with breadth of gene expression. Alu elements were also progressively more abundant as one considered more and more highly expressed genes while the densities of LINE-1 elements and several other repeats were negatively correlated with magnitude of gene expression. We propose that Alu elements constitute a cellular defense that protects genes against the repressive effects of other types of repeats.

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Recent Advancement of H-Invitational Database, An Integrated Database of the Human Transcriptome. *T. Imanishi[1], C. Yamasaki[1], Y. Fujii[1,2], Y. Yamaguchi-Kabata[1], T. Itoh[1,3], T. Gojobori[1,2,4], H-Invitational Consortium.* 1) Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan; 2) Japan Biological Information Research Center, Japan Biological Informatics Consortium, Tokyo, Japan; 3) National Institute of Agrobiological Sciences, Ibaraki, Japan; 4) Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Shizuoka, Japan.

On behalf of the H-Invitational Consortium, we present recent advances of H-Invitational Database (H-InvDB; <A HREF="http://www.h-invitational.jp">http://www.h-invitational.jp/), an integrated database of human transcriptome based on extensive annotation of human full-length cDNA (FLcDNA) clones. The latest release of H-InvDB (release 2.0, in preparation) drastically increased the number of FLcDNA clones registered by 37%, and contains annotation of 56,419 FLcDNAs. The number of loci predicted by mapping these FLcDNA sequences onto the human genome also increased by 21% to 25,585 loci. The database consists of descriptions of their gene structures, alternative splicing isoforms, functional non-protein-coding RNAs, functional domains of proteins, predicted subcellular localizations, metabolic pathways, predictions of protein 3D structure, mapping of SNPs and microsatellite repeat motifs, co-localization with orphan diseases, gene expression profiles, and comparisons with mouse full-length cDNAs in the context of molecular evolution. All the data in H-InvDB are shown in two main views, the cDNA view and the Locus view, and five auxiliary databases with web-based viewers; DiseaseInfo Viewer, H-ANGEL, Clustering Viewer, G-integra and TOPO Viewer, and provided as flat files and XML files. H-InvDB is a product of the H-Invitational project, an international collaboration to systematically validate human genes by analysis of a unique set of high quality FLcDNA clones using automatic annotation and human curation under unified criteria. This unique integrative knowledge-based platform for conducting *in silico* data mining represents a substantial contribution to resources required for the exploration of human biology and pathology.

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Gene mining: gene relevancy networking for complex human diseases. *X. Li[1,2], W. Jiang[1], L.H. Wang[3], Q.K. Wang[4], S.Q. Rao[1,4].* 1) Department of Bioinformatics, Harbin Medical University, Harbin 150086, China; 2) Department of Computer Science, Harbin Institue of Technology, Harbin 150001, China; 3) Department of Immunology, Harbin Medical University, Harbin 150086, China; 4) Departments of Cardiovascular Medicine and Molecular Cardiology, Cleveland Clinic Foundation, OH 44195, USA.

We have witnessed tremendous progress in elucidating the functional relationships between thousands of genes using high-dimension genomic data (e.g. microarrays), but less in mining the specific genetic networks relevant to the molecular pathogenic pathways for complex human diseases. In this study, we attempted to develop a robust data-mining-oriented alternative to unravel disease relevancy networks to complement the current model-based epistasis analysis utilized in human genetics. We designed a frequency measure (called adjusted frequency vote) to evaluate the strength of the relationships between the disease-relevant genes and to characterize their behaviors and topology within the disease-relevant gene forest. The empirical significances of the identified genetic relationships were determined by permutation tests. We have validated the proposed approach by building the colon-cancer relevancy networks using a publicly available microarray dataset. The results demonstrated that the disease-relevancy networks are the intricate interplays of the genes that are involved in the biological processes of cell proliferation, cell apoptosis, cell differentiation, mitogenesis and immunity. This study represents a pioneering and systematic endeavor to probe the underlying genetic complexities for complex human diseases.

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Lamin A/C and emerin mutations in Emery-Dreifuss muscular dystrophy disrupt Rb-MyoD pathways.

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Mutations of the Lamin A/C gene cause a large variety of human genetic disorders. We tested the transcriptional perturbation model using 125 human muscle biopsy mRNA profiling dataset containing 13 diagnostic groups. We show that two muscular dystrophies involving the nuclear envelope are highly related to each other (autosomal dominant EDMD [Lamin A/C], and X-linked recessive EDMD [emerin]). This data was consistent with Lamin A/C mutations causing similar downstream transcriptional perturbations as the Lamin A/C-interacting protein, emerin, and with the identical clinical symptoms in these two forms of EDMD. We used a multi-group bio-informatic method to define specific transcriptional pathways perturbed in Lamin A/C patients and emerin patients. We identified highly specific disturbances of a transcriptional regulatory pathway involving Rb, MyoD, Lamin A/C, and CREBBP/p300. Our data was consistent with a block in the transfer of Rb/MyoD-associated acetylation-deacetylation complexes during the differentiation of myoblasts to myotubes, and relative failure of appropriate timing and activation of downstream MyoD targets needed for differentiation. Consistent with this model, we found a coordinately regulated change in nuclear envelope components during muscle regeneration in vivo, with LaminB1-LAP1-LBR-emerin expressed during myoblast proliferation, and LaminB2-LAP2-LaminA-emerin coordinately induced during myoblast differentiation. We present a molecular model for transcriptional perturbations caused by Lamin A/C and emerin mutations, where lack of appropriate Lamin A/C - emerin - Rb - MyoD interaction and coordinated acetylation states leads to incorrect timing of exit from cell cycle and terminal differentiation of myogenic cells.

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A Novel Method Combining Linkage Disequilibrium Information and Imputed Functional Knowledge for tagSNP Selection. *R. Rochat[1, 2], L. de las Fuentes[2], V.G. Dávila-Román[2], G.D. Stormo[3], C.C. Gu[1].* 1) Division of Biostatistics, Washington University, St. Louis, MO; 2) Cardiovascular Division, Washington University, St. Louis, MO; 3) Department of Genetics, Washington University, St. Louis, MO.

The application of high density SNP maps in genetic studies has introduced problems of prohibitive genotyping costs and inflated false discovery rates. To attenuate this effect, selection strategies have been developed to choose subsets of representative SNPs (tagSNPs) for genotyping and analysis. Existing methods take two distinctive approaches to reducing dimensionality in SNP data for tagSNP selection. One approach is to focus on the biological property inherent to each SNP (Zhao et al. Human Mutation 2004, 23:534-9) and select those that are more likely to bear relevant functionality. The other approach utilizes the underlying linkage disequilibrium (LD) data structure among SNPs and selects by factor analysis (FA) those that are informationally representative (Nyholt et al. American Journal of Human Genetics 2004, 74:765-769). Methods assessing only functional information ignore LD data structure in the SNPs, whereas the FA approach neglects the biological significance of the SNPs. We propose a method using a weighted factor analysis (WFA) model that combines the strengths of both the functional and the informational approaches. We applied this method to highly dense SNPs found in cardiovascular candidate genes via re-sequencing by the SeattleSNPs program (NHLBI PGA), and compared our results with those derived by other popular tagSNPs selection strategies. The findings have been encouraging. For example, in the candidate gene PPAR-, from 78 SNPs in the European sample, tagSNPs selected by the WFA method seems to be more robust against varying minor allele frequencies (MAF), and consistently picked SNPs that were missed by focusing on function or LD structures alone. We will present a complete report of the analyses of SNPs by WFA in selected candidate genes re-sequenced by the SeattleSNPs PGA.

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Screening of mRNA sequences for alternative ORFs: potential effects on the complexity of the proteome and gene regulation. *C. Schoenbach[1], I.V. Kurochkin[1], A. Konagaya[2], M.S. Ali[3].* 1) Immunoinformatics Research Team, RIKEN Genomic Sciences Center, Yokohama 230-0045, Japan; 2) Advanced Genome Information Technology Research Group, RIKEN Genomic Sciences Center, Yokohama 230-0045, Japan; 3) Indian Institute of Technology, New Delhi 110016, India.

In general translation is initiated at the first AUG codon that is flanked by a favorable context sequence called Kozak consenus. However mechanisms such as leaky scanning and re-initiation through an internal ribosome entry site can initiate translation at a secondary AUG codon. We systematically examined translation initiation under the aspect of alternative translation using mouse cDNAs with complete coding sequence information extracted from GenBank. Predicted alternative open-reading frames (2,444 ORFs) candidates that shared at least 400 nucleotides with the GenBank-derived ORF (known ORF) were computationally categorized and annotated according to the type of ORF-overlap, Kozak context, AUG distance, cross-species conservation, protein motifs and solubility and potential similarity to known disease genes. The rule-based procedure yielded 1,597 alternative ORFs of which 288 were assigned a high probability of being translated. Among those, candidates with homology to human proteins and changes in protein properties are of particular interest. For example, one candidate transcript encodes in the known ORF a zinc finger motif-containing protein and a hypothetical protein in the alternative ORF, starting 184 nucleotides downstream of the known ORF. The protein encoded by the alternative ORF is tissue-specific and has been experimentally confirmed using antibodies. Translation of alternative ORFs not only increases the complexity of the proteome but implies new control mechanisms (i.e. transcriptional activation/repression) with implications for inducible processes such as immune response activation.

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Gene expression profiles of fragile X cells: comparison with normal controls and effect of 5-aza-2-deoxycytidine treatment. *P. Chiurazzi[1], C. Bernardini[2], A. Terracciano[1], C. D'Ambrosio[3], B.A. Oostra[4], P.J. van der Spek[5], G. Neri[1].*
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Fragile X syndrome is caused by transcriptional inactivation of the FMR1 gene and absence of the FMRP protein in affected patients. We investigated the expression profile of 18400 transcripts, including 14500 well-characterized human genes, in lymphoblastoid cell lines established from fragile X patients and normal male controls. We used the Affymetrix GeneChip Human Genome U133A 2.0 array and analyzed the transcriptional activity of two unrelated male patients with fragile X syndrome and a full mutation with 250-350 CGG repeats. The arrays were read with the Scanner 3000 and data were analyzed with the GCOS software and then with GeneSpring 7.2. Fragile X and control cell lines have consistently different expression profiles, which may prove useful in the identification of proteins interacting with and genes regulated by FMRP. Furthermore, we treated all cell lines (3 fragile X and 2 control) with 1 M 5-aza-2-deoxycytidine (5-azadC) for 7 days, extracted total RNA and prepared cDNA. We first tested the reactivation of the FMR1 gene in the fragile X lines by real-time RT-PCR, which reached 12%, 37% and 70% of normal controls, respectively. Then we prepared the cRNA probes and hybridized more arrays. We thus identified a set of genes up- or downregulated by the 5-azadC treatment in both fragile X and control cells, and also characterized the reactivation effect of 5-azadC on those genes that were differentially expressed between fragile X and normal males. Finally we checked the gene expression profile of the lymphoblastoid cell line (5106) with an unmethylated full mutation derived from a rare individual with an active FMR1 gene and normal intelligence. This work was supported by grant GGP030202 from Fondazione Telethon to G.N.

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Annotation of Genetic Polymorphisms in Drug-Metabolizing Enzymes. *C. Chiang[1], Z. Gu[1], S. Cai[1], R. Charlab[1], K.D. Lazaruk[2], A. Levitsky[1], C. Wan[1], T. Harkins[2], D. Ingber[1].* 1) Celera Genomics, 45 West Gude Drive, Rockville, MD 20850; 2) Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404.

Many polymorphisms within the genes of drug-metabolizing enzymes (DME) have been shown to alter drug responses in individuals. A DME genotyping platform would allow researchers to screen for these polymorphisms and may serve as an aid to determine individualizing treatment choice. However, a comprehensive catalog of DME polymorphisms is lacking, presumably due to the challenges in facing inconsistent nomenclatures and the high-degree homology of DME-coding genes. Here we report a bioinformatics process to annotate DME polymorphisms based on the data collected from both public and proprietary databases. Our process involves mapping the flanking sequences of polymorphic sites to genome assemblies, clustering DME polymorphisms, and assigning functional classification to the polymorphisms based on their relative positions to DME proteins. Ambiguous results were inspected and resolved manually by experts. Each unique cluster was assigned to a Celera accession number (CV), which represents one non-redundant polymorphic site. Several locus-specific allele nomenclature committees (e.g., Human Cytochrome P450 Allele Nomenclature Committee, http://www.imm.ki.se/CYPalleles) have been maintaining standard allelic designations, which are often haplotypes consisting of a set of polymorphic sites. To help researchers identify the polymorphisms reported by these committees, we assigned standard allele nomenclature to CVs by a pipeline that extracts and calculates the coordinates of DME polymorphisms on the reference sequences designated by the committees. DME alleles were assigned to CVs if their locations and bases both match. We have so far annotated more than 3,000 protein-coding SNPs for 218 DME-related genes.

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Custom Chip Definition Files For Affymetrix GeneChip Expression Microarrays. *R.J. Clifford, J. Zhang, R.P. Finney, K.H. Buetow.* National Cancer Inst, Bethesda, MD.

Affymetrix GeneChips are widely used for the analysis of gene expression, and many experiments performed with the HG-U95 and HG-U133 chip sets are now in the public domain. Probes on these arrays were designed against clusters from UniGene builds 95 and 133, respectively. In the time since array design human genome annotation has greatly improved and the UniGenes used for probe design have been refined. mRNA and EST sequences have been added to or removed from some clusters; other clusters have been split or merged. As a result, roughly 10% of probes on these microarrays hybridize to transcripts from multiple loci while another 10% do not match their target genes. Correct annotation of the HG-U95 and HG-U133 microarrays is critical for the analysis of gene expression experiments using these "legacy" platforms.

To address this need we have created custom Chip Description Files (CDFs) for the Affymetrix HG-U95A and HG-U133 microarrays. In our CDFs non-specific probes and mis-targeted probes are excluded from downstream analysis. To take advantage of the latest genome annotation, probes are grouped into sets based on NCBI Entrez Gene loci rather than the original UniGene clusters. If probes from more than one Affymetrix GeneChip probe set recognize the same gene, they are combined into a single probe set in the custom CDF. To examine gene expression in novel ways we have created three additional types of Chip Description Files: 1) CDFs containing probes sets that assay alternative splicing events, 2) CDFs that contain only probe sets located at the 3' ends of transcripts, and 3) CDFs containing probe sets that assay the expression of families of related genes.

Our custom CDFs are designed for use with the BioConductor statistical software package, which allows gene expression to be analyzed using a variety of algorithms such as MAS5 and RMA. We will demonstrate the advantage of using custom CDFs for gene expression by comparing results obtained from custom CDFs with those from traditional Affymetrix CDFs.

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Transcriptome analysis of a transgenic murine model of anxiety overexpressing the neurotrophin-3 receptor (NTRK3). *M. Guidi, I. Sahún, A. Amador-Arjona, M. Gratacòs, M. Dierssen, Y. Espinosa-Parrilla, X. Estivill.* Genes & Disease Program, Center for Genomic Regulation, Barcelona, Catalonia, Spain.

Neurotrophins and their receptors are essential for the development of the vertebrate nervous system by promoting neuroblasts survival, whereas in the adult brain they modulate neurite patterning and synaptic plasticity. Neurothrophic tyrosine kinase receptor 3 (*NTRK3*) is highly expressed in the *locus coeruleus* (LC) and its ligand neurothrophin-3 has trophic effects on noradrenergic LC-neurons. Changes in *NTRK3* expression might be implicated in the aetiology of anxiety disorders by inducing an overactivity of the noradrenergic system. A transgenic mouse overexpressing *NTRK3* (TgNTRK3) was generated. TgNTRK3 mice show an anxiety-related behaviour and an increase in the number of noradrenergic cells in the LC. The aim of this work is to study the consequences of *NTRK3* overexpression at the transcriptome level. Two transgenic murine lines were analysed. Total RNA was extracted with Trizol Reagent and DNase-treated RNA was used for microarray analysis (Agilent Mouse 22k-Oligo-Array) and RT-PCR expression studies. Developmental and postnatal stages were examined for TgNTRK3 expression, which was detected from embryonic stage E13 on. Microarray analysis was performed in cortex and hippocampus from 6 wild-type and 6 transgenic adult mice. We observed alterations in the expression of several cell signalling molecules and phosphatydilserine decarboxylases as well as a slight deregulation of some neuropeptides. Interestingly, a 3-fold downregulation of the thyroxine-binding protein (transthyretin) and a 1.3-fold downregulation of the thyroid hormone receptor interactor 10 (*Trip10*) were detected in the hippocampus, indicating a possible alteration of the thyroid hormone regulation in the transgenic mice. Some of the deregulated genes have been implicated in the modulation of fear and anxiety responses, in the regulation of the noradrenergic system or in panic and other psychiatric disorders. These results are consistent with the behavioural-histological phenotype previously observed in TgNTRK3, supporting the hypothesis of *NTRK3* being involved in anxiety disorders.

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Transcriptional profiling for detection of a gene signature in Rett Syndrome. *M.S. INAN[1], N. Kaya[1], M. Asyali[1], N. Sakati[2], M. Nester[2], P. Ozand[1,2].* 1) KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTER DEPARTMENT OF GENETICS, RIYADH; 2) KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTER DEPARTMENT OF MEDICINE, RIYADH.

Discoveries of a characteristic gene signature in various human disorders may help to reveal molecular mechanisms that responsible for the aggressive behavior of some of the diseases with possible translational implementation. Transcriptional profiling in various human disorders is an important tool to understand molecular mechanisms underlying a disease or syndrome. Herein, we performed whole gene expression analysis to identify a characteristic signature in on two families of multiple Rett patients using HU133 plus 2.0 Affymetrix GeneChips containing more than 47000 transcripts. Consistently over- and underexpressed genes in Rett patients as compared to normal individuals were found in various biological pathways. Several genes of interest that are differentially expressed between Rett patients and normal individuals were identified and confirmed with Real Time PCR. These genes will be presented with their biological functional groups such as signal transduction, metabolism, development, neurogenesis, cell growth, and regulation of transcription. These observations could help understanding the mechanisms of Rett syndroom and finding the pathways possibly involved in manifastation of Rett syndroome.

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***LRRK2* mutation screening in 100 families with autosomal dominant Parkinsons disease.** *J.M. Kachergus[1], I.F. Mata[1], J.P. Taylor[1], S. Lincoln[1], J. Aalsy[2], T. Lynch[3], M.M. Hulihan[1], R.A. Hauser[4], R.M. Wu[5], C. Lahoz[6], Z.K. Wszolek[1], M.J. Farrer[1].* 1) Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Department of Neurology, St. Olav's Hospital, Trondheim, Norway; 3) Department of Neurology, Mater Misericordiae University Hospital and Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; 4) Departments of Neurology, Pharmacology and Experimental Therapeutics University of South Florida and Tampa General Healthcare Parkinson's Disease and Movement Disorder Center ,Tampa, FL; 5) Departments of Neurology, Pediatrics and Medical Genetics, College of Medicine, National Taiwan University, and National Taiwan University Hospital, Taipei, Taiwan; 6) Servicio de Neurología, Hospital Universitario Central de Asturias, Oviedo, Spain.

In the last seven years mutations in six genes (*SNCA, PRKN, PINK1, DJ1, MAPT* and *UCHL-1*) have been linked to familial parkinsonism. We have identified mutations in a seventh gene, *LRRK2*(Leucine-rich repeat kinase 2; PARK8:OMIM609007), as responsible for autosomal dominant Parkinsons disease (PD). *LRRK2* maps to chromosome 12q12 (GenBank: AY792511) and encodes a large, multifunctional ROCO protein. Objective: To determine the contribution of *LRRK2* mutations in affected probands of kindreds with a family history of parkinsonism . Methods: All 51 exons were sequenced in 100 probands in which PD segregates as an autosomal dominant trait and in which DNA from at least two affected family members was availiable. The mutations identified were verified by ABI assay-on-demand probes in ethinicity-matched control samples and mutation segregation analysis was also performed. Semi-quantitative multiplex PCR was also used in order to rule out possible whole gene multiplications or deletions. Results: A total of 26 coding variants were identified, seven apparent pathogenic mutations, three of which are novel. The other 19 variants may be relevant to disease susceptibility or severity. The position of mutations within the gene, and the protein domains affected suggest aberrant kinase activity may underlie disease pathogenesis.

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Expression Changes Associated with the Tumor Suppressor Protein, Menin. *Y. Ji, E. Novotny, N. Prasad, J. Crabtree, A. Elkahloun, S. Chandrasekharappa.* Genome Technology Branch, NIH/NHGRI, Bethesda, MD.

Loss or inactivation of the MEN1 gene leads to multiple endocrine neoplasia type 1 (MEN1), an autosomal dominant disorder characterized by multiple tumors of the parathyroid, anterior pituitary and entero-pancreatic endocrine tissues. The amino acid sequence of MEN1 encoded protein, menin, provides little clues as to its function. In mouse models, homozygous loss of Men1 alleles results in embryonic lethality (E11.5-E12.5) suggesting importance of menin in early development. However, upon loss of the remaining Men1 allele as they age, heterozygous mice develop endocrine tumors resembling the human MEN1 condition. Menin, a nuclear protein, has been shown to interact with several proteins including transcription factors, JunD, SMAD1, 3 and 5, NFkb and MLL. To gain insight into the basic biological function of menin, we sought to identify target genes whose expression is altered in the absence of menin. For this we generated four wildtype (WT) and four null mouse embryonic fibroblast (MEF) cell lines from E9.5 embryos. Loss of menin does not appear to alter growth in these MEFs. RNA samples from WT MEFs were compared with RNA from null MEFs using high-density (20K cDNA) arrays to explore global expression changes. In addition, we looked at the menin-associated expression changes by expressing menin in Null MEFs lacking menin. We found that Fibulin-2 (Fbn2) and heat shock protein 25 (Hsp25) were abundantly expressed in the presence and absence of menin, respectively. Among several genes that showed significant changes were genes encoding adhesion/signaling/extra-cellular matrix proteins. Expression of several of these transcripts was verified by quantitative-PCR. Menin-mediated transcriptional regulation was evaluated using promoter for Cd24a in Luciferase reporter assays. The Cd24a promoter activity in Null MEF cell lines was suppressed by expressing wild type menin but not by mutant menin. The importance of expression changes in Cd24a and other genes in understanding the role of menin in development and tumorigenesis, if any, needs to be explored.

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Proteomic Analysis of gastric carcinomas. *H. Kurosaki[1], R. Nishigaki[2,3], M. Osaki[1,4], M. Hiratsuka[3], T. Toda[2], H. Ito[4], T. Inoue[3], M. Oshimura[1,3].* 1) Dept. Biomed. Sci., Tottori Univ. Grad. Sch. Med. Sci., Yonago, Japan; 2) Proteomics Collabo. Res. Group, Tokyo Metro. Inst. Geront., Itabashiku, JAPAN; 3) Dept. Hum. Genome. Sci., Tottori Univ. Grad. Sch. Med. Sci., Yonago, JAPAN; 4) Div. Organ Pathol., Dept. Microbiol. Pathol., Tottori Univ. Fac. Med., Yonago, JAPAN.

Although genetic alterations in proto-oncogenes, tumor-suppressor genes, cell cycle regulators, and cell growth factors have been implicated in the process of human gastric carcinogenesis, the principle carcinogenic mechanisms are not fully clarified. In this study, we used a proteomic approach to search for genes that may be involved in gastric carcinogenesis and that might serve as diagnostic markers. We identified 9 proteins with increased expression and 13 proteins with decreased expression in gastric carcinomas. The two most notable groups included proteins involved in mitotic checkpoint (MAD1L1 and EB1) and mitochondrial functions (COX5A, ECH1, and CLPP). This suggested that there are links between dysfunctions in these processes and gastric carcinogenesis. We also observed the differential expression of CYR61 and HSP27 proteins in gastric carcinoma, whose expression is known to be altered in other types of tumors. Furthermore, the study identified proteins whose function in gastric carcinomas was previously unsuspected and that may serve as new molecular markers for gastric carcinomas. Importantly, immunohistochemical analyses confirmed that the levels of expression of MAD1L1, CYR61, and HSP27 were altered in gastric carcinoma tissues. Therefore, our study suggested not only that the proteins identified in this study can be useful diagnostic markers but also that a proteomics-based approach is useful for developing a more complete picture of the pathogenesis and function of gastric carcinomas.

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Genomic Copy Number Detection with Microsphere-Based Suspension Hybridization. *H.L. Newkirk[1, 2], M. Miralles[1, 2], P.K. Rogan[1, 2], J.H. Knoll[1, 2].* 1) Childrens Mercy Hospitals and Clinics School of Medicine Childrens Mercy Hospitals and Clinics; 2) School of Medicine University of Missouri-Kansas City.

A microsphere-based suspension hybridization assay has been developed for high throughput detection of changes in genomic copy number. This assay allows for the direct analysis of whole genomic DNA extracted from residual fixed cell pellets and nick translated with biotin-dUTP. Single copy (sc) probes ranging in length from 60 to 2300 bp (100 bp average; Rogan et al Genome Res 11:1086, 2001) from ABL1 (chromosome 9q34), TEKT3 (17p12) and HOXB1 (7p15) were conjugated to spectrally-distinct polystyrene microspheres (Molecular Probes). Reactions were hybridized and detected using streptavidin-phycoerythrin conjugate and analyzed by flow cytometry. Using multiplex hybridization assays, 5 ABL1 deletions in patients with chronic myelogenous leukemia were confirmed by comparison of mean fluorescence intensities of chromosome 9q34 probes with the HOXB1 control (2 copies per diploid genome). The intensities of the chromosome 9 probes were significantly reduced in 5 deletion patients relative to the control probe. Similar experiments were performed with three trisomic 9 cell lines and in four duplication positive patients with Charcot-Marie-Tooth Type 1a disease (CMT1a). Using HOXB1 as an internal control, the assay distinguished 3 vs 2 alleles of chromosome 9 and 17 probes in the abnormal cell lines, where the mean fluorescence intensity of the chromosome 9 and 17 probes was about one third higher than for the control probe. Reproducibility studies for all patient samples yielded mean fluorescence intensity ratios that consistently distinguished copy number differences between the control probe and ABL1 or CMT1a probes. Unlike oligonucleotide bead hybridization assays, prior amplification of locus-specific target DNA was not required because the increased length and single copy composition of the hybridization probes increase the specificity required for direct detection of homologous genomic target sequences.

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Expression profiling of osteochondrodysplasia causative genes across the human growth plate. *E. Mougey, K. Mann, B. Maguire, J. Sylvester, R. Olney.* Nemours Biomedical Research, Nemours Children's Clinic, Jacksonville, FL.

The growth plates of long bones are located near each end of the bone and are responsible for the majority of linear growth. The growth plate can be divided into four functional regions or zones based on the characteristics of the chondrocytes that compose them. The recognized zones are (beginning from the articular end and progressing in the direction of differentiation): reserve, proliferative, pre-hypertrophic, and hypertrophic. A fifth zone, the perichondrium is located in apposition to the growth plate at its circumferential surface. The progression of growth plate chondrocytes through these different regions, and hence growth, is regulated by an exquisite interplay of signaling pathways. Defects in these signaling pathways that affect growth are manifested as osteochondrodysplasias. Currently, there are more than 200 known osteochondrodysplasias that have been divided into 33 clinically distinct groups, and seven distinct molecular-pathogenic groups. As of 2003, the molecular defect was known for slightly less than half of all osteochondrodysplasias. Acquisition of the expression profile of the long bone growth plate would be an important first step towards understanding its intricacies. Through a combination of laser microdissection, linear mRNA amplification, and Affymetrix U133 plus 2.0 GeneChip analyses, our laboratory has examined the expression profile of the five long bone growth plate fractions from two normal patients. Out of a possible 54,000 probe sets, the average number called positive by MAS 5.0 for all five arrays is 12,193 for patient 1 and 18,454 for patient 2. The average of the homologous inter-array correlations for the $\log_2$ expression levels is 0.910.01. Here we present the long bone growth plate expression profiles of expressed genes known to cause osteochondrodysplasias.

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Loci of shared segmental aneuploidy in the genomes of healthy and mentally retarded subjects detected by Array-CGH. *J.K. Ploos van Amstel, M.J. Eleveld, P.F.R. Hochstenbach, M. Poot.* Dept Medical Genetics, Univ Medical Ctr, Utrecht, Netherlands.

Using a 3,700 BAC array we performed Array-CGH on 131 children suspected of chromosomal disorders and 21 of their unaffected parents. Per individual we found on average statistically significant aberrant signals with 35 BACs. In 6% of our patients some of these signals represented pathogenetically relevant aberrations. In the remainder of our subjects these aberrant signals appeared independent of clinical symptoms and occurred with a frequency of 2% to 49%. In the patient and in the parent subset of our study population the same BACs occurred with similar frequencies as loci of shared segmental aneuploidy (SSA). In total more than 657 BACs indicated loci of SSA, which covered in total 2.8% of the euchromatin. Approximately 22% of loci of SSA occurred as duplications only, 35% as deletions only, and 44% as both deletions and duplications. The genomic distribution of loci of SSA varied widely among autosomes, with equal frequencies among Giemsa-light and Giemsa-dark bands. Approximately 34% of loci of SSA contained no genes at all, 8% contained only enzymes, and the remainder mixtures of genes from different classes, including numerous transcription factors. We conclude that loci of SSA occur frequently in the general population. The segregation of SSA from healthy parents to affected children, suggests that they have no apparent major clinical consequences.

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Whole genome microarray analysis of gene expression in Prader-Willi syndrome. *D.C. Bittel[1], N. Kibiryeva[1], S. Sell[2], T. Strong[2], M.G. Butler[1].* 1) Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) University of Alabama, Birmingham, AL.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, hypogonadism, mental deficiency and hyperphagia leading to early childhood obesity caused by loss of paternally expressed genes located in the 15q11-q13 region. A paternal 15q11-q13 deletion (Del) is found in about 70% of PWS subjects, uniparental maternal disomy (UPD) 15 in 25% and an imprinting defect in 3%. We used whole genome microarrays (Affymetrix U133A Plus 2.0 Array) to compare gene expression from actively growing lymphoblastoid cells from nine young PWS adult males (6 with Del, 3 with UPD) and four obese comparison males. As expected, there was no evidence of expression of paternally expressed genes (e.g., *SNRPN*) in the PWS cells. Of more than 47,000 genes examined, 22,151 met the inclusion criteria and 64 were overexpressed ($\geq$ 2X) and 386 were underexpressed ($\leq$ 2X) in the PWS cells relative to comparison cells. Of more highly expressed genes in PWS, four (*BCL2L11, CD47, CREM, UCHL1*) were assigned to neurological development/function, two (*CREM, DHRS9*) to endocrine function and two (*CD47, DHRS9*) to lipid metabolism. Of genes in PWS with reduced expression, 19 were related to neurological development/function, five to endocrine function, seven to lipid metabolism and 11 to respiratory function. GABA receptor genes (A5, B3) in 15q11-q13 had expression reduced by 1.7X in PWS relative to controls consistent with previous paternal bias observations. *HTR2B* a serotonin receptor and genes involved with controlling eating behavior (*POMC, CART*) along with an oxytocin receptor, all outside of 15q11-q13, had reduced expression while an 8X increase in expression of the orexin gene which stimulates eating were found in PWS compared to controls. The number of candidate genes with detectable altered expression is relatively small consistent with a recent report of microarrays in a PWS mouse model (Stefan et al., 2005). In addition, 61 genes were more highly expressed ($\geq$ 2X) in the PWS-UPD group relative to the PWS-Del group and 65 genes were more highly expressed in the PWS-Del group compared to PWS-UPD.

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The diverse impacts of mobile elements on the human genome. *P. Deininger[1], S. Gasior[1], V. Perepelitsa-Belancio[1], T. Wakeman[2], B. Xu[2].* 1) Tulane Cancer Center and Dept of Epidemiology, New Orleans, LA; 2) Dept. of Genetics, LSUHSC, New Orleans, LA.

Long INterspersed Element-1 (L1) is probably the only active human autonomous retroelement, and it also drives the retroposition of the non-autonomous elements, Alu and SVA. The proposed mechanism of insertion for L1 suggests that cleavage of both strands of genomic DNA is required in a process termed target-primed reverse transcription (TPRT). In an effort to look at the double-strand breaks (DSBs) made by the L1 and how the cell is responding to them, we found that L1 expression leads to a high level of DSBs in DNA relative to the level of retrotransposition insertions. This level is several orders of magnitude higher than needed for the number of retrotransposition events needed. This was demonstrated using immunolocalization of -H2AX foci, phospho-ATM foci and direct measurements of DNA breaks with the COMET assay. Similar to its role in mediating DSB repair in response to radiation, ATM kinase appears to be required for L1 retrotransposition. DSBs produced by endogenously expressed L1 likely contribute to chromosomal rearrangements in the human germ line and somatic tissues independent of insertional mutagenesis and represent a major new endogenous source of DSBs. This is likely to include increased levels of recombination, particularly Alu/Alu recombination. The cell responds to these DSBs with induction of DNA repair responses, but they may overwhelm the cell and become toxic. These impacts are likely to vary depending on cell type in which L1 is expressed and to be greatly enhanced in many tumors where L1 expression is deregulated. It is also likely to be influenced by environmental influences and the genetic background of different individuals.

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Submicroscopic genomic aberrations: RPGR and OTC contiguous gene deletion revealed by array CGH. *K.A. Rauen[1], R.C. Gallagher[2], P.D. Cotter[1], A.L. Estep[1], G.M. Enns[2], M.F. Marmor[2].* 1) University of California, San Francisco, CA; 2) Stanford University, Palo Alto, CA.

Retinitis pigmentosa (RP) is a progressive retinal dystrophy that is genetically heterogeneous. X-linked RP (XLRP) accounts for approximately 10% of affected individuals. Of three XLRP loci on Xp, one of these (RPGR) is in close proximity to the gene for ornithine transcarbamylase (OTC). OTC deficiency is the most common urea cycle defect. Each of these disorders presents with severe clinical symptoms in affected males and a spectrum of symptoms in carrier females, from asymptomatic to severely affected. We present a family with both XLRP and OTC deficiency and examine the clinical utility of a genome scanning microarray to detect a possible aberration affecting the X chromosome. The propositus is a 45-year-old woman noted to have a 30 year history of reduced vision and retinal "scars", with recent complaints of night blindness and field loss. Ophthalmologic evaluation demonstrated decreased visual acuity. The fundi showed vascular narrowing and mild waxiness of the discs. There was diffuse thinning of the retinal pigment epithelium in both eyes. Full-field electroretinograms were abnormal and cone b-waves were markedly delayed. Her 23 year-old daughter had a similar ophthalmic history and findings, and the propositus had a son who died of a hyperammonemic episode as an infant. Testing of the propositus revealed OTC deficiency by protein challenge. The family history was highly suggestive of a contiguous gene deletion involving chromosome Xp, but a high resolution karyotype was found to be normal. Further evaluation by array CGH demonstrated 4 BACs with single copy loss indicating a 3.8 Mb submicroscopic deletion of chromosome Xp11.4-p21.1, encompassing the RPGR and OTC genes. The same deletion was also found in her daughter. This study demonstrates that families with XLRP need to be thoroughly evaluated including a detailed family history to look for possible signs and symptoms of OTC deficiency and that array CGH is a powerful tool for the identification and characterization of chromosome abnormalities not readily identified by conventional cytogenetic analysis.

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2D proteomicmap of amniotic fluid supernatant sample. *A. Mavrou[1], A. Kolialexi[1], P. Karamessinis[2], A. Antsaklis[3], M. Fountoulakis[2], G. Tsangaris[2].* 1) Med Gen, Aghia Sophia C H, Athens Univ Medical Sch, Athens, Greece; 2) Division of Biotechnology, Center of Basic Research, Foundation of Biomedical Research of the Academy of Athens, Athens, Greece; 3) 1st Department of Obstetrics & Gynecology, Athens University School of Medicine, Athens, Greece.

Proteomic analysis, combining two-dimensional electrophoresis and mass spectrometry has the potential for a wide range of applications in biological and medical sciences, as protein screening in tissues obtained from healthy and diseased conditions can be performed for the detection of drug targets and diagnostic markers. Conventionally, amniotic fluid samples (AF) are routinely used for prenatal diagnosis of a wide range of fetal abnormalities. Proteomics have already been applied in the analysis of tissues from fetuses with Down syndrome, in order to detect differences in their protein profile as compared to the normal ones and to determine possible diagnostic tools. A detailed two-dimensional protein database for the normal human AF cells has not as yet been reported. In the present study we constructed the two-dimensional protein database of the normal human AF supernatant. Fifteen AF samples from women carrying normal fetuses were analysed by two dimensional gel electrophoresis. A mean of 412 spots per gel were analysed and protein identification was carried out by MALDI MS and MS/MS. We constructed a two-dimensional protein map comprising of 152 different gene products. The majority of the identified proteins are enzymes, secreted proteins, carriers and immunoglobulins. Twelve hypothetical proteins are also icluded. The normal AF supernatant proteome map is a valuable tool for the study of aberrant protein expression and the search for proteins as possible markers for the prediction of abnormal fetuses.

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Use of whole genome amplification and comparative genomic hybridization to detect chromosomal copy number alterations in small amounts of tumor tissue. *V. Rusakova, L. Nosek, E. Mueller, T. Favello, T. Rull, R. Valdes-Camin.* R&D, Sigma-Aldrich, Saint Louis, MO.

Cancers often induce structural genetic alterations creating either gene loss or gene amplifications. These changes are difficult to measure due to the limited amount of biopsied clinical material. We have established that DNA array comparative genomic hybridization (CGH) can be accurately used to identify genome-wide copy number abnormalities of nanogram DNA samples after amplification with Sigmas GenomePlex Whole Genome Amplification Kit (WGA). We present data for both control and cancer samples that demonstrate the fidelity and reproducibility of WGA for the determination of genome-wide ploidy differences. This example demonstrates the WGA kit generates sufficient DNA for CGH analysis without introduction of sequence representation bias. The combination of whole genome amplification and CGH using gDNA extracted from cancer patient material has the potential for permitting the studying of DNA from small cancerous loci, and may help to identify genomic markers for early diagnosis.

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Screening to find genes causing cleft lip and palate. *K. Osoegawa[1], R. Pfundt[2], J. Staaf[3], E. Lammer[1], J. Murray[4], A. Borg[3], E. Schoenmakers[2], P. De Jong[1].* 1) Childrens Hosp Oakland Research Inst, Oakland, CA; 2) Dept. Human Genetics, University Medical Centre Nijmegen, The Netherlands; 3) Dept. Oncology, Lund University, Sweden; 4) Pediatrics and Biological Sciences University of Iowa, IA.

Human bacterial artificial chromosome (BAC) libraries have played a critical role for construction of physical maps and genome sequencing. We have developed a high-resolution overlapping-BAC collection of >32,000 well-characterized clones, spanning the entire human genome. We have used PCR-amplified DNAs from these BACs to create the array comparative genomic hybridization (CGH) slides with a detection resolution for copy number variation of about 70 kbp. Cleft lip with or without cleft palate (CL/P) are common structural birth defects. We have begun to survey the genome for candidate gene regions by searching for deletions/duplications using DNA sample derived from CL/P patients. In our first control experiment, approximately 8% of the clones (2,500) generated ratios outside the arbitrary low and high cut-off points (0.3) of log2 (T/R) values. We consider these data points to indicate the false positive rate. The second control experiment used male DNA with a known deletion (11.3 Mb) on chromosome 9 and female DNA with a known deletion (21.9 Mb) on chromosome 7, labeled with Cy5 and Cy3, respectively, and co-hybridized on a single 32k array slide. As expected, we were able to detect the sex mismatch as copy-number variation for chromosomes X and Y, and revealed the chromosome -9 and -7 deletions. Our control experiments gave us the confidence that we will be able to identify genomic changes as small as 300 kb. We have identified large numbers of deletions and duplications using DNA from 11 CL/P patients as test sample against reference DNA. We have identified novel large duplication on chromosome 8p21.3-8p12 (8.8 Mb) and deletion on chromosome 8q23.1-8q24.12 (11.2 Mb) from one of the samples. Some copy number changes were identified from multiple samples. We are in the process of analyzing lager number of samples, creating catalog of duplicated and deleted regions and list of genes located in the regions.

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Sequencing the chimpanzee Y chromosome to understand its evolutional and biological characteristics. *Y. Kuroki[1], A. Toyoda[1], H. Noguchi[1,2], T.D. Taylor[1], H.S. Park[3], S.H. Choi[3], Y.S. Lee[3], D.W. Kim[3], D.S. Kim[3], T. Itoh[4], T. Yamada[2], S. Morishita[2], M. Hattori[1,5], S. Sakaki[1], A. Fujiyama[1,6].* 1) RIKEN Genomic Sciences center, Yokohama Kanagawa, Japan; 2) School of Frontier Sciences, Kashiwa, Chiba, Japan; 3) Genome Research Center KRIBB, Daejeon, Korea; 4) Mitsubishi Research Institute Inc., Chiyoda-ku, Tokyo, Japan; 5) Kitasato University, Sagamihara, Kanagawa, Japan; 6) National Institute of Informatics, Chiyoda-ku, Tokyo, Japan.

Human chromosome Y is known to have male-specific characteristics and a peculiar genomic structure including abundant chromosomal-specific repeats, large-scale duplications, and complicated chromosomal rearrangements. To understand the evolutional and biological characteristics of the Y chromosome, we have started a comparative sequence analysis between the human and chimpanzee Y chromosomes. We first constructed a physical map of chimpanzee Y chromosome, and then started sequencing clone by clone. To date, we have completely finished the sequencing of 100 BAC and fosmid clones; the total non-redundant length of the sequenced region is about 11 Mb. This region contains 27 genes which are derived from human Yp and Yq. Many structural differences such as rearrangements, insertion-deletions, and duplications have been found so far in this study. In addition, we have also identified chimpanzee-specific regions or the regions which are very low in conservation between human and chimpanzee by end sequence analysis of chimpanzee Y-specific fosmid clones. By comparing the genomic sequence and the clone mapping patterns from FISH analysis, we can identify the conserved or diverged regions along the chromosome. We will perform a population analysis for these differences to distinguish the polymorphisms from the species-specific differences. The diversity between species or individuals will become clearer with this study. These results will be helpful to understand the human-specific characteristics and the evolutional aspects of the peculiar Y chromosome.

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Definition of genomic structural variation and mutation by segregation analysis: the 8p23.1 beta-defensin region as a model system. *E.J. Hollox[1], A. Doria[2], J.A.L. Armour[1].* 1) Inst Genetics, Univ Nottingham, Nottingham, United Kingdom; 2) Joslin Diabetes Center, Harvard Medical School, Boston, MA.

Several recent studies have emphasised the widespread nature of large-scale genomic polymorphism, such as inversions and copy number variation. Analysis of such regions is limited both by their intrinsic variability and the standard technology, which is most suitable for invariant single copy genomic sequences. Here we show that analysis of recombination and segregation in well-characterised families can yield important information on the organisation and behaviour of such complex regions. As a model system we analysed the 8p23.1 region, which shows common copy number polymorphism, common inversion polymorphism, frequent pathological chromosomal rearrangements, and has been implicated by linkage in several diseases such as type 2 diabetes, deafness and schizophrenia. With additional marker information, we analysed several families from the CEPH cohort, as well as five families with comprehensive genetic marker information, cytogenetic information on inversion status, and dosage information using multiplex amplifiable probe hybridisation (MAPH). We identified recombinations within 8p23.1 that caused alteration of beta-defensin copy number, confirming this as the mutational mechanism for loss or gain of beta-defensin repeats and allowing estimate of the mutation rate can also be made from this data. We also show that inversion carriers can be identified from segregation patterns, and that recombination within the inverted region shows that the beta-defensin repeats are polymorphic in chromosomal location, with repeats consistently present at one site but variably present/absent at the other site.

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Gene-Expression profiles in human nasal polyp tissues from aspirin intolerant asthma patients. *T. Sekigawa[1], Y. Sakamoto[1], A. Tajima[1], S. Matsune[2], I. Inoue[1].* 1) Div Genetic Diagnosis, Inst Medical Sciences, Tokyo, Japan; 2) Department of Otolarynology, Faculty of Medicine, Kagoshima University.

In a subset of asthmatic patients, aspirin and several other non-steroidal anti-inflammatory drugs (NSAID) that inhibit cyclooxygenase (COX) enzymes induce severe asthmatic attack generally termed aspirin intolerant asthma (AIA). In 198 unrelated AIA patients, we identified prostaglandin E2 receptor subtype 2 gene is a susceptibility to AIA (Hum Mol Genetics 2004), however, the etiology of AIA can not be approached simply by genetic factor. Symptoms of AIA are characterized by aspirin sensitivity, asthma, and nasal polyposis known as Sameters triad. It is known that rhinosinusitis or nasal polyp is frequently observed before the onset of asthmatic attack. Therefore, hyperplastic rhinosinusitis associated with aspirin intolerance should play important roles in the development of AIA. In the current study, we determined the gene-expression profiles of nasal polyp tissues from 13patients including six aspirin intolerant asthma and five eosinophilic sinusitis and two chronic sinusitis using Agilent Human Oligo 1A ver2 comprising approximately 18,000 unique DNA oligonucleotides for gene-expression detection. We identify 52 genes whose expression were significantly different (2 fold up-regulated 45 genes or 0.5 fold down-regulated 7 genes were defined as differential expression, T-test P-values ware less than 0.05) between AIA and eosinophilic sinusitis. For clustering purpose, nonnegative matrix factorization (NMF) was applied and the expression profile was classified into three groups. One group was particularly rich in genes participating arachidonic acid metabolic cascade and inflammatory response. These groups would help to identify susceptibility and pathophysiolosical pathway underlying AIA. The study will now being proceeded to disclose expression profiles and individual genes related to AIA that would illustrate the bio-pathway and genetic abnormality of AIA.

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The Identification and Functional Analysis of Conserved Noncoding Sequences of *SCN1A*, *SCN2A*, and *SCN3A*.

M. Martin, A. Escayg. Department of Human Genetics, Emory University, Atlanta, GA.

Two voltage-gated sodium channel alpha subunits, *SCN1A* and *SCN2A*, have been implicated in different epilepsy subtypes. Mutations in both genes lead to Generalized Epilepsy with Febrile Seizures Plus (GEFS+). *SCN2A* mutations also lead to Benign Familial Neonatal-Infantile Seizures (BFNIS), while *SCN1A* dysfunction frequently leads to Severe Myoclonic Epilepsy of Infancy (SMEI). Many *SCN1A* mutations are predicted to abolish channel function, suggesting that reduced channel expression leads to altered neuronal excitability. To determine if mutations in transcriptional regulatory regions of *SCN1A* or *SCN2A* contribute to disease, we conducted comparative sequence analysis to locate potential regulatory regions. Comparative genomics was conducted across a 1.1Mb region on human chromosome 2q24.2 containing a cluster of neuronal voltage-gated sodium channels, *SCN1A*, *SCN2A*, and *SCN3A*. The genomic organization of the locus was conserved across the human, mouse, dog, and chicken genomes. Twenty-two conserved noncoding sequence blocks greater than 100bp in length and 75% sequence identity were discovered from orthologous regions of the human, mouse, and dog genomes. Six noncoding regions were conserved across the human paralogs, *SCN1A*, *SCN2A*, and *SCN3A*. Nineteen of these conserved noncoding sequences (CNSs) were also present in the chicken genome. The CNSs at the *SCN1A* locus were examined by luciferase reporter gene assays for their ability to alter transcription. One CNS (CNS3) significantly increased the transcription of the SV40 promoter. The possible RNA secondary structures of each CNS were determined by *in silico* analysis. CNS7 formed a stable hair-pin-loop structure characteristic of miRNAs, illustrating that a subset of CNSs potentially represent noncoding RNAs.

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Standardization of RNA Quality Assessment for better reproducibility and reliability of gene expression experiments using the RNA Integrity Number (RIN). *M. Valer[1], S. Lightfoot[2], R. Salowsky[1].* 1) Agilent Technologies, Waldbronn, Germany; 2) Agilent Technologies, Palo Alto, Germany.

Good RNA quality assessment is considered one of the most critical elements to obtain meaningful gene expression data via microarray or real-time PCR experiments. Advances in microfluidic technology have improved RNA quality measurements by allowing a more detailed look at patterns of RNA degradation via the use of electrophoretic traces. However, the interpretation of such electropherograms still requires a certain level of experience and can vary from one researcher to the next. The RNA integrity number(RIN) algorithm is introduced to assign a user-independent integrity number to each RNA sample. The RIN has been developed using neural networks by teaching this algorithm with a large number of RNA integrity data. The RIN score, based on a quality numbering system from 1-10 (in ascending quality), facilitates the classification of RNA samples to be used in the context of the gene expression workflow. In order to correlate microarray results with RIN and other alternative RNA quality measures, samples degraded for different times, where hybridized on microarray chips together or in parallel experiments. The results clearly show an important influence of the RNA sample integrity state as determined by the RIN towards the accuracy and noise levels in the array experiments..

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Identification of an additional 5% of protein-coding genes in the human genome. *T.D. Taylor[1], Y. Totoki[1], T. Yada[2], Y. Sakaki[1], T. Takeda[1].* 1) Comp Exper Sys Biol Grp, RIKEN Gen Sci Ctr, Yokohama, Japan; 2) Dept Intell Sci & Tech, Kyoto Univ, Kyoto, Japan.

Ab initio gene-finders have essential potentialities for exhaustive rare gene predictions. The remarkable feature of ab initio gene-finders is their high sensitivity, especially at the DNA level. We introduce a method for identifying novel genes expressed at an extremely low level by using our DIGIT gene-finding program. DIGIT finds genes exhaustively and effectively by combining gene predictions from multiple ab initio gene-finders such as FGENESH, GENSCAN and HMMgene. DIGIT discards many false positive exons predicted by the other gene-finders and yields remarkable improvements in specificity without lowering sensitivity.

We applied DIGIT to human chromosome 11 and predicted 68 novel gene loci whose genomic regions do not overlap with Ensembl genes or human mRNAs in GenBank. To verify these candidate genes, RT-PCR was performed using primers prepared based on the predicted exon sequences with a single-strand cDNA library prepared from various human tissues. In the first round of RT-PCR, we could scarcely identify the PCR products for most of the predicted gene candidates, suggestive of extremely low expression in the tested tissues or uncertainty of the gene prediction. We then conducted a second round of PCR using nested-PCR primer sets with the diluted RT-PCR products.

We finally identified 39 novel gene loci which transcribe 74 distinct mRNAs. The success rate of our experimental verification was 57%. The identified genes were expressed at an extremely low level, and were likely absent from the cDNA libraries were used in various high-throughput full-length cDNA and EST sequencing projects. 73% of the novel transcripts were specifically expressed, suggesting that these gene products physiologically are operating in a tissue-specific manner.

Based on our analysis of the entire genome, we predict that the number of human protein-coding genes may be as much as 5-10% higher than current estimates.

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Selecting SNPs for genetic association studies based on the genome-wide patterns of linkage disequilibrium inferred from the HapMap Project data. *C.R. Scafe, H. Isaac, F.M. De La Vega.* Applied Biosystems, Foster City, CA, USA.

The design of genetic association studies using single-nucleotide polymorphisms (SNPs) requires the selection of subsets of the variants providing high statistical power at a reasonable cost. SNPs must be selected to maximize the probability that a causative mutation is in linkage disequilibrium (LD) with at least one marker genotyped in the study. Reports have shown significant variation of LD across regions and populations, implying that efficient marker selection should consider the empirical patterns of LD. The HapMap project performed a high-density, genome-wide survey of genetic variation with about a million SNPs typed in four populations, providing a rich resource to inform the design of association studies. A number of strategies have been proposed for the selection of SNPs based on observed LD. These include the metric LD maps, which provide a means to place markers according to the local LD context, and the selection of haplotype tagging SNPs. Statistical power calculations are important, as irreproducible associations are partially due to under-powered studies. Integrating all these methods and annotations can be challenging: the algorithms required to develop LD maps, select tagging SNPs, estimate power, etc, are complex to deploy, and all the necessary data and annotations are deposited in disparate databases. To simplify this process, we developed the SNPbrowser Software, a freely available tool to assist in the LD-based selection of markers for association studies. In its current version, this stand-alone application provides fast query capabilities and swift visualization of SNPs, gene annotations, power, haplotype blocks, and LD map coordinates derived from the HapMap project data. Wizards implement several common SNPs selection workflows and SNP tagging algorithms. Optimal sets of tagging SNPs provide reduction in genotyping burden by 55-75%, depending on the metric/threshold used. These sets have been screened for their conversion potential to either TaqMan SNP Genotyping Assays or the SNPlex Genotyping System, expediting the set-up of genetic studies with an increased probability of success.

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Transport conditions impact DNA yield from buccal cell specimens collected in epidemiologic studies. *M. Gallagher, C. Sturchio, M. Honein.* CDC, Atlanta.

The National Birth Defects Prevention Study (NBDPS) is one of the largest case control studies of birth defects ever conducted in the United States. The goal of this ongoing multi-center study is to identify environmental and genetic risk factors for birth defects. Information on environmental risk factors is collected through a maternal interview, and DNA is collected from the infant and both parents for evaluation of genetic risk factors. Cheek cell (buccal) specimens are obtained because of their low cost and ease of use for sampling infants without the direct involvement of health-care workers. However, unlike whole blood, buccal specimens yield lower amounts of DNA that is often of poorer quality. Initially, NBDPS study participants received buccal cell collection kits containing sterile cytology brushes in sealed plastic tubes. After collecting the specimens, participants were instructed to place the brushes back into the plastic tube and mail them to the NBDPS Center. Although the plastic tubes provided a convenient storage container, laboratory data from several NBDPS Centers suggested that buccal specimens that are kept in a moist environment have lower DNA yields which is worsened at elevated temperatures such as those occurring in summer months. Beginning in August 2003, the packaging for the cytology brushes was changed to non-resealable peel pouches. Use of this type of packaging allows the brushes to air dry during return transport through the mail. To evaluate the impact of the change in the buccal collection protocol, DNA yields from a subset of specimens obtained from one Center were determined using real time PCR. Mean yields of human DNA from buccal brushes stored in plastic tubes compared to brushes stored in peel pouches were 1.09 g (range 0-8.2) vs. 2.17 g (range 0.73-9.99) for specimens obtained from mothers, 1.15 g (range 0-5.85) vs. 2.01 g (range 0.07-17.6) for fathers, and 0.76 g (range 0-6.22) vs. 2.06 g (range 0.12-10.5) for infants. The use of peel pouches for temporary storage of buccal brushes significantly increased yield of human DNA obtained from specimens from all family members; the largest increase in DNA yield was observed in specimens obtained from infants.

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ABRF/DSRG DNA Sequencing Troubleshooting Guide. *H. Escobar[1], D. Adam[2], M. Detwiler[9], S. Hardin[8], J. Kieleczawa[7], D. Needleman[3], R. Pershad[4], P. Schweitzer[5], G. Wiebe[6].* 1) University of Utah, Salt Lake City, UT; 2) University of British Columbia, Vancouver, BC; 3) USDA/ARS Eastern Regional Research Center, Wyndmoor, PA; 4) University of Texas M.D. Anderson Cancer Center, Houston, TX; 5) Cornell University, Ithaca, NY; 6) Max Planck Institute of Molecular Cell Biology & Genetics, Dresden, Germany; 7) Wyeth Research, Cambridge, MA; 8) University of Houston, Houston, TX; 9) Roswell Park Cancer Institute, Buffalo, NY.

The importance of automated DNA sequencing as a genetic tool is evidenced by the considerable expansion of facilities performing such analyses. Coupling this with the rapid growth of the technology itself creates a vast network of operators presenting varying experiences with instrument platforms and chemistries. Yet despite recent advances in the automated DNA sequencing process, periodic problems associated with instrumentation and reaction processes continue to exist. The establishment of a public database would allow for a continually evolving resource, which grows and expands with the technology, utilizes the experiences of a wide variety of contributors, and offers the unique advantage of always being current. The aim of the ABRF/DSRGs (Association of Biomolecular Resource Facilities/DNA Sequencing Research Group) web-based DNA Sequencing Troubleshooting Resource is to provide a forum for users to share information, through the creation of a searchable, comprehensive troubleshooting guide for the DNA sequencing community. With the launch of the live web-site scheduled for the end of 2005, we will present a snapshot of the resource as it exists presently.

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Whole genome quantitative association studies implicate SNPs in Oxysterol-Binding Protein 2 (OSBP2) in the regulation of cholesterol metabolism. *J.C. Engert[1], L. Oksanan[2], B. Ge[2], M. Lemire[2], S. Gurd[2], K. DesBiens[3], D. Shah[3], S. Bailey[1], J. Faith[2], T.J. Hudson[1,2], T. Pastinen[2].* 1) Department of Human Genetics, McGill University, Montréal; 2) McGill University and Genome Quebec Innovation Centre, Montreal; 3) McGill University Health Centre, Montréal.

We, and others, have previously demonstrated heritability of mRNA levels in the pedigrees of CEPH families. We have now performed a large-scale survey of quantitative variation associated with specific SNPs that were included in the HapMap project. If we examine quantitative traits in the unrelated parents of these trios (the cell lines from which was derived the HapMap data), these experiments are the equivalent of performing a whole genome quantitative association scan in 60 individuals. Specifically, an association analysis was conducted between the SNPs from the Caucasian (Utah) samples of the HapMap project and the mRNA level of the low-density lipoprotein receptor (LDLR). The LDLR mRNA was measured in the lymphoblast cell lines of 30 CEPH trios under cholesterol deprivation conditions. Previous work has shown that these cells can be an appropriate cell model for the regulation of certain lipid metabolic pathways. In addition, one of the key responses to cholesterol deprivation is an increase in LDLR and this response is preserved in lymphocytes. Approximately 600,000 SNPs from the HapMap project were available, and we tested the approximately 400,000 that had an allelic frequency of at least 0.025. We have 80% power to detect an SNP that contributes to 20% of the variance. This approach allowed us to identify the Oxysterol-Binding Protein 2 (OSBP2) as a candidate gene for the control of cholesterol metabolism. The OSBP2 gene contains 14 exons, and spans 200 kb of chromosome 22q12. We observed two SNPs that that seem to contribute to the variation in the level of LDLR mRNA after cholesterol deprivation. Combining the detailed genetic information provided by the HapMap from cell lines with biomedically relevant quantitative phenotypes collected from these same cell lines should demonstrate great utility. Thus, we believe this technique will be applicable to complex traits and associated biochemical pathways.

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Pathway analysis reveals mitochondrial OXPHOS defects and increased apoptosis in T-cells from individuals at-risk for Type 1 Diabetes. *P.A. Jailwala[1], J. Waukau[1], G. Duinkerken[2], B.O. Roep[2], D. Eckels[1], L. Meyer[1], M. Hessner[1], J. Rovensky[1], H. Khoo[1], S. Milosavljevic[1], S. Jana[1], S.W. Guo[1], S. Ghosh[1].* 1) Max McGee National Research Center for Juvenile Diabetes, Medical College of Wisconsin, Milwaukee, WI; 2) Dept. of Immunohaematology & Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.

Type 1 diabetes mellitus (T1DM) is a T-cell mediated disease in which the insulin-producing pancreatic beta cells are destroyed. In this study, we examined gene expression in response to anti-CD3 stimulation, in two types of CD4 T cell clones: a GAD65-specific clone from a pre-diabetic and a Hepatitis C-specific clone. Microarray analysis was carried out with human gene chips containing 9,128 genes. Co-hybridization (6 replicates) for the three time points (1, 2 and 4 hours) was carried out, comparing *anti-CD3 stimulation* to *no stimulation*. After data filtering and normalization, we performed mixed-model ANOVA and bootstrapping (99% CI), to identify truly differentially expressed (DE) genes. The results show that there are no DE genes in common between GAD and HepC across 3 time-points. To test for gene-sets that might by systematically altered in each clone, we then used pathway over-representation analysis, which determines whether there is an over-represented pathway in the DE category compared to the proportion of genes in the same pathway on the whole microarray chip. Results show that the GAD clone undergoes perturbation of genes involved in oxidative stress/OXPHOS at the 1hr time-point. At 2hrs, this previous mitochondrial activity is usurped by apoptosis genes in the DE list. We identified 13 down-regulated anti-apoptotic genes and 20 up-regulated pro-apoptotic genes in GAD. Furthermore, CASP3 up-regulation indicates triggering of the caspase cascade in response to cytochrome-c release from the mitochondria. We are currently confirming expression of these apoptosis/OXPHOS genes by RT-PCR in the GAD clone. Finally, we confirmed increased apoptosis in at-risk individuals in a larger sample size through an apoptosis assay on a heterogeneous population of anti-CD3 stimulated CD4+CD25- T-cells.

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Genome-wide Analysis of Hereditary Prostate Cancer Families Using High Density Microarray-based SNP Genotyping. *W. Liu[1], B.L. Chang[1], S.L. Zheng1[1], T. Li[1], J. Sun[1], A. Tolin[1], W.B. Isaacs[2], J. Xu[1].* 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

The existence of more than 10 million DNA variations, primarily single nucleotide polymorphisms (SNPs), allows for fine dissection of the associations between genetic variants and complex diseases. A number of commercially available microarray based systems, with different resolutions, are currently available for whole-genome genotyping (Syvanen, 2005) using diploid genomic DNA from different sources. In an effort to screen for DNA sequence variants associated with prostate cancer, we utilized the GeneChip Human Mapping 100K microarray which contains 116,204 SNP probes on two chips (Hind 240 and Xba240) to assay genomic DNA isolated from blood and corresponding lymphoblastoid cell lines. Four large hereditary prostate cancer (HPC) families with a total of 25 subjects have been analyzed thus far. The average successful SNP call rates using genomic DNA from blood for Hind 240 and Xba 240 were 99.15% and 99.25%, respectively, while the average SNP call rates using genomic DNA from cell lines were 98.65% and 98.71%, respectively, for these two chips. The average concordance of genotyping between blood and cell lines was 98.57% with a standard deviation of 0.4%. The majority of the non-concordant genotypes between blood and cell lines were due to non calls instead of different calls. In summary, the data from our study reveals that excellent SNP call rates can be achieved using Affymetrix GeneChip Human Mapping 100K microarrays. Secondly, the data provide evidence that we can accurately genotype SNPs to facilitate genome-wide association studies. Finally, cell line DNA can be reliably used as a substitute for blood DNA for genome-wide genotyping analysis.

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Evol-genomic: a software tool for translational genomics. *M.A. Zapala[1], G. Hsiao[2], K.N. Chang[3], N.J. Schork[1].* 1) Biomedical Sciences, UCSD School of Medicine, La Jolla, CA; 2) Department of Bioengineering, UCSD, La Jolla, CA; 3) Division of Biological Sciences, UCSD, La Jolla, CA.

The inference that genes influencing phenotypes in model organisms also influence similar phenotypes in humans is complicated by a number of evolutionary phenomena. We interrogated factors that may mediate gene expression differences between humans and mice using DNA sequence data, synteny maps, gene/protein structural information, and expression data, in an effort to facilitate understanding of factors that may complicate translatability of genetic analysis findings in mice to humans. The Homologene database was downloaded from NCBI, syntenic data was downloaded from Ensembl and breakpoint data was downloaded from TIGR. Multiple regression was used to combine scores of sequence similarity, synteny, genetic distance and the ratio of non-synonymous to synonymous SNPs. A comparison of mouse to human kidney gene expression data was used as a training set to obtain a regression model. Only orthologous genes that were scored as Present in both mouse and human samples were included. The regression model attempted to predict the absolute difference in gene expression between human and mouse. The model produced an R value of 0.564 indicating a relationship with a third of the variation predicted by the model. An ANOVA was performed (F Statistic 0.05) showing that the variation explained by the model was significant. Using gene expression data from human and mouse heart tissue, a cross-validation of the model was performed with 0.11 shrinkage indicating moderate universality of the model. There has yet to be an inclusive assessment of multiple evolutionary factors which might lead to insights into the reliability of translating gene expression across mice and into human populations. Evol-genomic fills this vacuum and provides bench scientists with a user-friendly intuitive tool that empowers them to gain knowledge into how evolutionary factors may have impacted their candidate genes. Evol-genomic is designed for users to input a set of candidate genes from their expression studies in mice and output a ranked list of genes whose expression is most likely to be preserved in humans.

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Genome-wide survey of gene copy number change spanning over 20 million years of human and primate evolution. *J. Sikela[1], L. Dumas[1], Y. Kim[2], A. Fortna[1], A. Karimpour-Fard[1], J. Hopkins[1], J. Pollack[2].* 1) Human Medical Genetics, Univ Colorado Health Sci Ctr, Aurora, CO; 2) Department of Pathology, Stanford University, Stanford CA.

We have previously applied cDNA array-based comparative genomic hybridization (aCGH) genome-wide across human and four great ape genomes and identified over 1,000 genes showing lineage-specific increases or decreases in copy number. We have now extended the breadth of this technology to compare genomes that are more closely related (i.e. within the human population) and those that are more distantly related (estimated divergence >20 MYA, e.g. between human and macaque/baboon).

Within-species comparisons used DNAs from 15 diverse human sub-populations covering three major continents. Among copy number polymorphisms (CNPs) identified and independently confirmed were two for adjacent genes (MTX-1 and GBA) in two African samples. A previous study of these genes found them to be CNPs particularly in African-Americans, consistent with our data. These CNPs were not found by three published genome-wide studies of human CNPs, suggesting that in certain cases, cDNA aCGH may identify CNPs not detected by other platforms.

Extension of cDNA aCGH to the macaque and baboon identified over 1400 genes that gave aCGH signatures indicative of lineage-specific copy number changes. These were specific to either macaque or baboon or shared by both, with similar numbers of increases and decreases. A number of genes gave strong positive aCGH signals indicative of pronounced lineage-specific copy number expansions.

Finally, because human is used as the reference in all comparisons, cDNA aCGH datasets from a wide range of species can be related to one another and gene copy number expansions that have occurred across large evolutionary time frames (>20 MY) directly identified.

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Highly efficient recombination-based methods for bacterial artificial chromosome fusion and mutagenesis. *B.L. Sopher, A.R. La Spada.* Lab Medicine and Center for Neurogenetics & Neurotherapeutics, Univ Washington, Seattle, WA.

To extend the utility of bacterial artificial chromosomes (BACs) for the study of gene function and disease modeling, we have developed: i) a simple bacteriophage-based methodology for recombining overlapping BACs into a single larger BAC, and ii) a new methodology for targeting "seamless" mutations into BACs. In the first method, overlapping sequence from the terminus of one BAC is cloned alongside a selectable marker and placed between unique sequence arms from the terminus of the other BAC to create a targeting construct. Two successive rounds of recombination-based cloning are then performed to fuse the two BACs into one contiguous BAC that encompasses both of their insert sequences. The robustness of this methodology is demonstrated herein by using it to obtain a 254 kb BAC containing the entire human androgen receptor (hAR) gene from two smaller BACs. In the second method, transient expression of three bacteriophage genes (exo, bet, and gam) to pop-in a targeting cassette is followed by RecA expression from the targeting vector itself to pop-out the vector backbone. This new "hybrid recombineering" method thus combines the strengths of bacteriophage and RecA based bacterial recombination systems, while avoiding their major weaknesses. Successful application of this method for introduction of a properly targeted 162 CAG repeat expansion into the hAR 254 kb BAC is shown. With "hybrid recombineering", we believe that the power and utility of the classical pop-in / pop-out approach - so commonly and efficiently employed in yeast for decades - can now be achieved with BACs.

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Insertion pattern analysis of transgenes in a transgenic mouse line by genomic walking. *O. Suzuki, M. Koura, K. Takano, Y. Noguchi, K. Uchio-Yamada, J. Matsuda.* Experimental Animal Models, Natl Inst Biomed Innovation, Ibaragi-Shi, Osaka, Japan.

Simple and accurate genotyping methods, especially zygosity check systems, are necessary for efficient use and management of transgenic animals as laboratory animals. For this purpose, we previously reported a simple and efficient method for genetic mapping and zygosity check of transgenes (Noguchi et al., Exp. Anim. 53:103-111, 2004). Here we report that our transgene mapping technique using genomic walking can be used for analysis of transgene integration patterns in transgenic mice. The technique revealed that a transgenic mouse line (GM1-sy#116) harbored inverted and direct tandem repeats of both intact and partial pCAGGS-based transgenes in the G2 region of the chromosome one. It is widely accepted that concatemers, mostly direct tandem repeats, are formed from vector constructs before integration into chromosomes. According to Bishops model (Reprod. Nutr. Dev. 36:607-618, 1996), the reason why direct-tandem repeats is more common than inversed tandem repeats is that concatemers are created by homologous recombination of circularly permuted linear molecules. In our case, however, the complicated chromosomal integration of transgenes might be caused by a simple end-joining of DNA constructs which might have been fragmented by exposure to a UV transilluminator during gel-purification as well as by nuclease digestion inside zygote pronuclei. Our results indicate the usefulness of genomic walking technique, and also suggest that care should be taken at preparation of vector constructs to avoid unexpected fragmentation prior to microinjections to zygotes.

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A genome-wide definitive haplotype structure determined using a collection of complete hydatidiform moles. *Y. Kukita[1], K. Miyatake[1], R. Stokowski[2], D. Hinds[2], K. Higasa[1], N. Wake[3], T. Hirakawa[3], H. Kato[3], T. Matsuda[3], K. Pant[2], D. Cox[2], T. Tahira[1], K. Hayashi[1].* 1) Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Perlegen Sciences Inc., Mountain View, CA; 3) Division of Molecular and Cell Therapeutics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.

We present a genome-wide definitive haplotype structure determined using a collection of 74 complete hydatidiform moles, each of which carrying the genome derived from a single sperm. The haplotypes consist of 280,000 common SNPs, genotyped by a high throughput array-based oligonucleotide hybridization technique. Comparison of haplotypes inferred from pseudo-individuals (constructed by randomized mole pairs) with those of moles showed minor errors in resolution of phases by the computational method. The effects of those errors on local haplotype structure and selection of tag SNPs are discussed. We also show that the definitive haplotypes of moles may be useful for elucidation of long-range haplotype structures, and should be effective ways to detect extended haplotype homozygosities that are indicative of positive selections.

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Detection of extremely rare mutations by multiple oligonucleotide mismatch PAP-A (MOM-PAP-A). *Z. Chen, J. Feng, S.S. Sommer.* Molecular Genetics, City of Hope National Medical Center, Duarte, CA.

Pyrophosphorolysis-activated polymerization (PAP) was developed to detect extremely rare mutations in a DNA pool with primers modified by adding a 3-terminal dideoxynucleotide. The theoretical selectivity of blocked primers is 1 part in 3.3×1011. However the practical selectivity of PAP is 1:104 to 1:105 because of misincoporation within the extension product from the opposing primer. Bi-directional PAP allele specific amplification (Bi-PAP-A) eliminates the bypass reaction using two opposing blocked primers with one nucleotide overlap at their 3 termini, and can selectively detect one copy of a single base substitution in 109 copies. However, the selectivity for detection of deletions, insertions, and indels is about one part in 104. Herein, we present multiple oligonucleotide mismatch PAP-A (MOM-PAP-A), an ultra-sensitive form of PAP for detection of mutations other than single base substitutions, including deletions, insertions, or indels. For MOM-PAP-A, both downstream and upstream primers are blocked and separated, resulting in an amplified segment of 100~300bp. One primer is mutant-specific and the 3 terminus is a few nucleotides away from the site of the deletion. The result is that the mutant-specific primer mismatches the wild type sequence at 2~7 nucleotides. The selectivity of MOM-PAP-A was examined using the epidermal growth factor receptor (EGFR1) gene as a model and the EGFR2 and F9 genes as controls. One molecule of the deletion or complex mutation can be detected per 50 microliter amplification reaction and there is no signal in the presence of >108 or >109 wild type molecules. In conclusion, MOM-PAP-A is the most sensitive way to detect rare mutations including deletions, insertions and indels. MOM-PAP-A can be applied to detect mutations in single cells because of its high sensitivity. MOM-PAP-A also can detect somatic mutations from surgically removed tumors in blood, allowing detection of minimal residual disease at orders of magnitude greater sensitivity than by conventional methods.

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Genetic Interaction between ionotropic and metabotropic serotonin receptors in antipsychotic response. *V. De Luca, R. Hwang, D.J. Mueller, C. Zai, J. Volavka, S.G. Potkin, H.Y. Meltzer, J.A. Lieberman, J.L. Kennedy.* Dept Neurogenetics, Univ Toronto, Toronto, ON, Canada.

Serotonin receptor genes have for many years been considered excellent candidate genes in the etiology of neuropsychiatric diseases. Defects in the in promoter regions could be cause of inherited disease, affecting the efficiency of RNA transcription. Clozapine is a potent antagonist of 5-HT2 and 5-HT3 receptors. Reynolds and colleagues (2002), demonstrate an association between antipsychotic drug induced weight gain and a promotor region polymorphism (C-759T) of the 5-HT2C receptor gene. In the only clozapine response study of the 5-HT3A gene, Gutierrez et al. (2002) found negative results with the C178T marker. We prospectively assessed a sample of 101 patients with DSM-IIIR diagnoses of schizophrenia using the Brief Psychiatric Rating Scale (BPRS) at baseline and following six weeks of clozapine treatment. The patients were categorized as responder and non-responder defined as 20% reduction of BPRS score. Single gene analysis produced a positive result for the 178T allele of 5HT2A predicting response (chi-square=6.52, 2df, p=0.03) and negative results for -759T (chi-square=2.58, 2df, p=0.27). A logistic regression analysis was used to examine the combined effect the variant alleles (-759T) and (178T). No statistically significant differences were found between the presence of two alleles combined (chi-square=2.918; 2df p=0.232) and clozapine response. Our failure to find positive results with gene-gene interaction may be attributed to sample ethnicity differences (African American and Caucasian). It is possible that the alleles at each polymorphism are in different linkage disequilibrium conditions among the ethnic groups. Further investigation is required to assess whether these polymorphisms may have a predictive role for clozapine response.

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Single molecule sequencing by synthesis. *S.N. Lapidus, P.R. Buzby, T.D. Harris, J.W. Efcavitch.* Helicos BioSciences Corp, Cambridge, MA.

We will present a technology which sequences true, non-amplified, single molecules of DNA, and will highlight its utility in complex disease association studies and heterogeneous tumor analysis. The platform is based on sequencing by synthesis, which provides sequence information from individual strands of DNA attached to a glass substrate and processed in parallel. Using DNA polymerase and fluorescently labeled nucleotides, single base incorporations are monitored simultaneously on multiple template strands by fluorescence microscopy. Our optical setup consists of a total internal reflection microscope and a cooled CCD camera, which is capable of imaging an entire substrate (30,000 fields) in 50 minutes. For disease association studies, high throughput targeted gene resequencing with this technology allows the discovery of rare genetic aberrations including point-mutations, insertions/deletions and amplifications. In tumor samples, the high coverage afforded by parallel sequencing reveals mutations as rare as 1%. Eventually, this technology promises to enable whole human genome resequencing directly from genomic DNA purified from 100 cell equivalents without amplification. 10X genome coverage will be achieved in days, reducing resequencing costs 1000X vs. conventional Sanger sequencing.

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Evaluating data in a high throughput SNP genotyping facility to maximize quality, cost, and efficiency. *C. Bark, E. Pugh, H. Hobbs, Y. Tsai, B. Craig, K. Hetrick, I. McMullen, J. Romm, M. Zilka, M. Barnhart, J. Goldstein, R. King, C. Boehm, M. Brehm, J. Gearhart, K. Doheny.* IGM, Johns Hopkins Univ, Baltimore, MD.

In recent years, SNP genotyping has risen in prevalence due to increases in scalability and information content, in conjunction with decreases in cost, laboratory resources and manual data review. However, the advances made in these areas have produced their own complications. The Johns Hopkins University has provided high quality microsatellite data to the scientific community over the last eight years and is dedicated to providing the same level of quality for its SNP genotyping services. Compared to microsatellite genotyping, per experiment, SNP genotyping methods have increased both the number of samples and loci processed. Because one experiment may include hundreds of samples for thousands or even hundreds of thousands of SNPs, a user or system error can have a large impact on overall data quality and cost. This has required that quality control methods be revised to focus on real time evaluation of data. Some of the methods used include; 1) Creating unique plate signatures through sample placement that allows incorrect sample transformations informatically or in the laboratory to be easily distinguished during the initial quality assurance stage. For standard marker panels, known CEPH controls are used. For custom marker panels, known genotypes for CEPH controls are compiled from public databases. 2) Safeguarding against sample plating and manifest errors by genotyping all DNA samples with two multiplexed microsatellite test panels containing 21 markers. 3) Using Spotfire DecisionSite and in house tools to visualize data trends in both standard and flexible formats. Problems can be identified and addressed closer to real-time. 4) Creating validated reagents and procedures for troubleshooting experiments to identify questionable operations, reagents and consumables in an expedited manner. These tools enable lab personnel to explore the data and to troubleshoot problems quickly and independently.

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Genotyping 500K SNPs on a pair of arrays. *R. Mei, H. Matsuzaki, H. Loi, M. Lu, K. Travers, J. Law, T. Berntsen, M. Chadha, X. Di, G. Liu, E. Hubbell, G. Yang, T. Webster, S. Cawley, G. Marcus, M. Shapero, S. Walsh, K. Jones.*
Affymetrix, Santa Clara, CA.

Genome-wide association analysis provides a powerful means of identifying the common variants that contribute to complex disease predisposition and drug response. However, this type of studies requires a dense set of SNPs across the genome and a tool to score the SNPs accurately and cost-effectively. We present a high throughput platform that enables the parallel genotyping of over 500,000 SNPs on a pair of arrays. Achieving this goal required a number of advancements in array technology and assay development, most notably shrinking feature sizes down to 5 ^{2}, reducing the feature numbers to 24 features per SNP and increasing target complexity to ~660 Mbases. Call rates across the 500,000 SNPs are > 98%, reproducibility is > 99.9%, and accuracy, as measured by inheritance in trios and concordance with the HapMap Project, is > 99.5%. The average inter-marker distance is 3.3 kb, and 80% of the genome is within 10 kb of a SNP. To assess the utility of the 500,000 SNPs for genome-wide association studies in various populations, we determined heterozygosity and allele frequencies and, more importantly, analyzed the extent of linkage disequilibrium (LD) in four populations, namely Yoruba in Ibadan (Nigeria), Japanese in Tokyo (Japan), Han Chinese in Beijing (China), and CEPH (Utah residents with European ancestry).

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GENOMIZER - An Integrated Analysis System for Genome-Wide Association Data. *A. Franke[1], A. Wollstein[1], M. Teuber[1], M. Wittig[1], T. Lu[3], P. Nürnberg[2], M. Krawczak[3], J. Hampe[1], S. Schreiber[1].* 1) Institute of Clinical Molecular Biology, Christian-Albrechts University Kiel, Germany; 2) Cologne Center for Genomics, University of Cologne, Germany; 3) Institute for Medical Informatics and Statistics, Christian-Albrechts University Kiel, Germany.

Genome-wide association analysis appears to be a promising way to identify heritable susceptibility factors for complex human disorders. However, the feasibility of large-scale genotyping experiments is currently limited by an incomplete marker coverage of the genome, a restricted understanding of the functional role of given genomic regions, and the small sample sizes used. Thus, genome-wide association analysis will be a screening tool to facilitate subsequent gene discovery rather then a means to completely resolve individual genetic risk profiles. The validation of association findings will continue to rely upon the replication of "leads" in independent samples from either the same or different populations. Even under such pragmatic conditions, the timely analysis of the large data sets in question poses serious technical challenges. We have therefore developed public-domain software, GENOMIZER, that implements the workflow of an association experiment, including data management, single-point and haplotype analysis, "lead" definition and data visualisation. We suggest that the use of this software will facilitate the handling and interpretation of the currently emerging genome-wide association data. The software is available for academic use at http://www.ikmb.uni-kiel.de/genomizer/.

This implementation was performed as a pilot project in the German GRID computing initiative MEDIGRID.

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STRP Genotyping Methods: Implementation of Cost Reduction and Quality Control Processes in a High Throughput Environment. *M. Zilka[1], J. Romm[1], C. Bark[1], C. Boyce[1], B. Craig[1], K. Hetrick[1], H. Hobbs[1], M. Brehm[1], C. Ongaco[1], A. Robinson[1], C. Boehm[1], J. Cahlik[2], K. Doheny[1].* 1) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Acme-Automation, Spring City, TN.

High throughput genotyping facilities require implementation of cost and time saving methods as well as strict quality controls. CIDR recently reduced its standard PCR volume from 5uL to 3uL using a script from Acme Automation for the Biomek 2000, which allows for a 3ul dispense into dry plates. This change alone resulted in a savings of 2.5 cents per genotype or over $275,000 per year. Savings are also accomplished by bulk PCR master mix reagent creation. The advent of improved spectral resolution over a wider dynamic range on ABs 3730 allowed for the optimization of large multiplexes of PCR primers. The multiplex primer pools and bulk mastermix are tested before use to ensure adequate performance. Before production begins on a project, CIDR runs a set of test markers to ensure the DNA is not contaminated, is amplifying well and to identify sample misidentification. Performance of each of our 404 markers is tracked automatically and checked periodically to quickly identify problems. 3730 runs are automatically monitored for failure and immediately requeued. Data analysis incorporates automatic flags at the genotype level for unexpected positive control values, more than 2 alleles, discrepant allele size and bin name values, and failure to review a poor quality genotype. CIDR also has automated systems for requeuing PCR marker failures and validating that all expected data is present as a project is finalized.

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Molecular response to acute shortening and lengthening exercise in human. *M.C. Kostek[1], D.J.R. Cuthbertson[2], M. Fedele[3], K. Esser[3], M. Rennie[2], YW. Chen[1].* 1) Genetic Medicine, Children's National Med Center, Washington, DC; 2) Department of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland;; 3) School of Biomedical Sciences, Graduate Entry Medical School, University of Nottingham, Derby City Hospital; 5School of Kinesiology, University of Illinois, Chicago, USA.

Phenotypic changes in size and maximal force production have been well characterized in skeletal muscle in response to stimuli. Furthermore, it is known that eccentric muscle contractions (EC) lead to greater increases in muscle hypertrophy and maximal force production than concentric contractions (CC). However, little is known about the molecular basis of these differences. In this study, we examined the changes in skeletal muscle gene expression in response to a single bout of EC and CC using expression profiling. Three healthy male volunteers, 25 5 y, underwent an acute bout of training of the quadriceps. Each subject performed CC to fatigue with one leg while the contralateral leg underwent the same number of EC. Muscle biopsies were taken before exercise and 3, 6, and 24 hours post exercise from each leg. RNA isolated from these samples was expression profiled using a custom Affymetrix MuscleChip microarray containing ~4,000 muscle specific transcripts. Differences in gene expression between the two types of contractions were compared at each time point using Welch t-test. We identified 57, 57 and 148 genes differentially regulated at 3, 6 and 24 h time point respectively. At the 3 and 6 h time point, half of the known genes differentially regulated between the EC and CC were involved in stress responses and gene regulation, while at 24 hours, mybfibrial and cell growth related genes in addition to stress response genes were dominant. One of the genes, cardiac ankyrin repeat protein, which was up-regulated at 24 hours was verified by Western blot (~30%) at the protein level. This study in conjunction with our previous studies demonstrated specific patterns of gene expression in response to a hypertrophy inducing stimulus. The data suggests that CC and EC can produce similar yet distinct patterns of gene expression.

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LINKAGE AND MUTATION ANALYSIS OF THE ZFHX4 GENE IN PATIENTS WITH CONGENITAL PTOSIS. *W. Chan[1], C. Andrews[1,3], E.C. Engle[1,2,3].* 1) Department of Pediatrics (Genomics), Children's Hospital Boston, Boston, MA; 2) Department of Neurology, Children's Hospital Boston, Boston, MA; 3) Harvard Medical School, Boston, MA.

Congenital isolated ptosis (CIP) is the most common eyelid anomaly and manifests as abnormal drooping of the upper eyelid with narrowing of the palpebral fissure. CIP is unilateral in ~75% of sporadic cases, and is more frequently bilateral when inherited. We previously mapped a CIP gene, PTOS1, to chromosome 1p32-p34.1. In 2000, McMullan et al identified a de novo balanced translocation 46, XY,t(1:8) (p34.3;q21.12) in a patient with bilateral CIP. The chromosome 1p breakpoint is 13 Mb outside the PTOS1 region, while the chromosome 8 breakpoint disrupts intron 4 of ZFHX4, a 10-exon zinc finger homeodomain transcription factor whose mouse homolog is expressed in embryonic midbrain and muscle. To determine if any of our CIP probands harbored mutations in ZFHX4, DNA was extracted using standard techniques from peripheral blood of 17 familial and 15 sporadic CIP study probands. Linkage analysis was performed by fluorescent PCR amplification of 6 polymorphic markers flanking the ZFHX4 region. ZFHX4 exons and flanking intronic sequences were PCR amplified and analyzed by DHPLC (Transgenomic WAVE). Variant amplicons were sequenced bidirectionally (ABI 3730 DNA Analyzer, Applied Biosystems). The structure of 12 of the familial cases permitted meaningful linkage analysis, and 9 of these pedigrees were consistent with linkage to ZFHX4. Therefore, ZFHX4 was sequenced in 14 familial and 15 sporadic probands. None of the 29 probands harbored ZFHX4 mutations. Eleven polymorphisms were identified. The absence of ZFHX4 mutations suggests that the reported balanced translocation may result in ptosis by a position effect of the chromosome 1 breakpoint on the unidentified PTOS1 gene.

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Molecular characterization of the canine factor VIII gene and duplicated sequences related to a common gene inversion mutation. *N. Tayebi, L. Wood, J.N. Lozier.* Laboratory of Hemostasis, Division of Hematology, CBER/FDA Rockville MD.

Hemophilia A (HA), the deficiency of the coagulation factor VIII (FVIII), is the most common sex-linked inherited bleeding disorder. It is cause by mutations in the large (186 kb and 26 exons) and complex FVIII gene on chromosome Xq28. The major identified mutation hot spots are in intron1 and intron 22 with 40 to 50% of severe HA cases arising from intron 22 inversions. The best characterized animal model of HA is the Chapel Hill HA dog, which has an inversion similar to the common human intron 22 FVIII gene inversion. To understand the mechanism of HA in the Chapel Hill hemophilia A dogs Better, we set out to determine the complete sequence of the normal dog FVIII gene. The RPC1-81 BAC library is derived from normal male Doberman Pinscher genomic DNA. BAC clones 291 M9, and 292 C4 from this library were shown to contain FVIII gene sequences by hybridization with dog FVIII exon probes. BAC clone 292 C4 contains exon 1-22 and BAC clone 314 O16 contains intron 21-exon 26. A third BAC clone, 291 M9 contains non FVIII sequence that is found in HA dog FVIII transcripts in the place of exons 23-26. All three BACs carry a factor 8 associated (F8A) sequence, which is the site of recombination in the common inversion mutation. High throughput sequencing established 14 contiguous fragments of sequence for BAC clone 292 C4. We have established overlaps between 10 of these fragments by PCR, subcloning and sequencing of overlap fragments from the 292 C4 BAC and dog genomic DNA. We have identified overlapping FVIII sequence in BAC clone 314 O16 beginning at intron 21. Comparison of our existing Doberman FVIII genomic DNA sequence with that FVIII genomic DNA sequence derived from the canine genome project (http://ensembl.bii.a-star.edu.sg/Canis_familiaris/) that used Boxer genomic DNA showed 99% homology between exonic DNA sequence but numerous intronic polymorphisms. Complete FVIII genomic sequencing will permit development of a rapid PCR diagnostic test for the common FVIII inversion in dogs.

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Vega: a bright new star in the genome browser firmament. *L.G. Wilming, J.L. Harrow, C. Amid, A. Frankish, R.C. Gibson, J.G.R. Gilbert, E.A. Hart, G.K. Laird, J.E. Loveland, J. Mudge, J. Rajan, H.K. Sehra, C.A. Steward, M.-M. Suner, D. Swarbreck, S. Trevanion, T. Hubbard.* Wellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridgeshire CB10 1HH, United Kingdom.

The Vega (VErtebrate Genome Annotation) web browser is an Ensembl derived system dedicated to browsing manually annotated genomic sequence, currently mostly human, mouse and zebrafish. Vega displays manual annotation performed by the Havana group at the Sanger Institute, as well as that produced by other genomic annotation groups (WashU GSC, Genoscope, JGI, Broad, IMB Jena). Manual annotation is better suited to accurate determination of tandem duplicated gene clusters and classes of genes like pseudogenes and (potentially) non-coding genes than automatic systems such as Ensembl. Additionally, polyA features, splice variation and nomenclature benefit from manual annotation.

Apart from the emphasis on manual annotation, Vega also differs from Ensembl in that the underlying genomic sequence assembly is more up-to-date as it is not bound to a fixed update cycle. Vega also shows unique data not found elsewhere, for example human chromosome 6 MHC haplotypes and the dog chromosome 12 MHC.

Here we present data on comparisons between Vega annotation of syntenic human and mouse regions. For example the significant re-arrangements that have taken place between human chromosome 9 (hs9) and a deletion region on mouse chromosome 4 (mm4) and between a disease related part of hs17 and mm11. In both cases groups of genes consecutive in one species are found in a significant different order on the other species owing to micro and macro rearrangements. We also observe gene duplications or genes unique to one species and gene clusters such as Krt, Krtap and Mup expanded in one species relative to the other. Other comparisons being explored are hs20 versus mm2, which contains the entire syntenic region of hs20, and mm4 versus hs1 and hs6. Vega will be able to display the annotation of any of these regions simultaneously in human and mouse through the MultiContigView interface.

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TaqMan siRNA assays by stem-loop RT-PCR. *R. Tan, C. Chen, K.J. Guegler.* Advanced Research and Technology, Applied Biosystems, Foster City, CA.

RNA interference (RNAi) induced by short interference RNA (siRNA) is a powerful tool for gene silencing. The short life of synthetic siRNA has limited its applications. Short hairpin RNAs (shRNAs) driven by polymerase III promoters have been investigated as an alternative strategy to stably suppress gene expression. Since the position at which Dicer RNase III cleaves a hairpin of shRNA is not well defined, a single shRNA molecule may generate several putative siRNA products at both sense and antisense orientations. We have recently developed a new real-time quantitation method termed stem-loop RT followed by real-time PCR for accurate and sensitive detection of microRNAs. We report here the use of this method to specifically quantify shRNA-derived siRNAs. Concordance between siRNA copy number and the level of target gene expression was observed in the shRNA vector-transfected cells. Our results have further demonstrated the accuracy and sensitivity of the looped-primer RT-PCR method in the quantitation of shRNA-derived siRNA products, and have also demonstrated that the profiles of shRNA-derived siRNA products depend on the structure and sequence of shRNA. The TaqMan siRNA assay is a valuable tool in optimizing the shRNA construct, monitoring the level of siRNAs, understanding the shRNA-based RNA interference (RNAi) pathway, and evaluating the stability and efficacy of shRNAs as a therapeutic agent.

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Allele-Specific PCR (ASPCR) Pre-screening for High-throughput Genomics. *A. Scott, J. Bodnar, J. Goldstein, G. Spangler.* Institute of Genetic Medicine, Johns Hopkins School of Medicine 600 N. Wolfe Street, Baltimore, MD 21287.

Background: Genotyping centers process hundreds of samples per day. Quality control measures for reagents, instrumentation, and procedures generally insure reliable results. However, sample mislabeling, or cross-contamination may occur before processing. It is essential that mistakes be discovered before analysis. We wished to develop an economical method to identify sample mislabeling associated with gender. The assay should be facile and add little time or expense to the overall genotyping process. Methods: Design of three primers using allele-specific parameters allowed amplification of the amelogenin gene to produce a single band for females (279bp) and two bands for males (279 and 159bp.) These were resolved by standard agarose gel electrophoresis. PCR product size determination and subsequent gender calls were performed automatically. Experimentation involved two phases 1) Condition testing to optimize the method, and 2) concordance testing to validate the assay. Demonstration of the amplification as a predictor of amplification success was demonstrated using a heparin titration, and exposure to DNaseI. Results: Optimization experiments produced an inexpensive and robust assay that could be performed in less than two hours with complete automation. A mock run reproduced and identified a pipetting error similar to that for which the assay was designed. Analysis of samples previously processed in a high-throughput facility identified an incorrectly labeled sample. Furthermore, performance of our assay gave an indication of sample performance on amplification-based SNP platforms. Conclusions: Confirmation of sample identity and DNA quality using our method is possible with little expense, negligible sample consumption, and little time added to the high-throughput genotyping process.

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Resequencing Process for the Discovery of Variants in Human Mitochondrial DNA. *Y. Lei, P. Ma, X. Chen, L. Leong, X. Lin, M. Rydland, P. Baybayan, C. Heiner.* Applied Biosystems, Foster City, CA.

Human mitochondrial DNA is present in large numbers in every cell in the body, and exhibits characteristics that make this molecule ideal for evolutionary studies, forensic analysis, and clinical genetic diagnosis. This DNA is a circular molecule that has 16,569 bp, encoding 37 genes for oxidative phosphorylation, two rRNAs genes and 22 tRNA genes. We have developed a complete resequencing workflow for mitochondrial DNA, and designed primers to cover the entire genome. Our primer design resulted in 46 amplicons, including 9 for the control region. Our workflow includes protocols for PCR amplification and cycle sequencing, and uses SeqScape software v2.5 for mutation detection and report generation. We tested our primer sequences and workflow by completely sequencing the mitochondrial DNA from 23 Coriell DNAs, obtaining high quality data in both orientations for at least 97% of the mitochondrial genome in all cases. We also discovered novel variants from samples when the sequences were compared to Mitomap Reference sequence. We will describe the details of our workflow and our results.

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High-throughput Alternative Splicing Quantification by Primer Extension and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. *C. Ding[1], R.M. McCullough[2], C.R. Cantor[3].* 1) Centre for Emerging Infectious Diseases, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region; 2) Program of Molecular and Cellular Biology, Boston University; 3) Center for Advanced Biotechnology, Boston University.

Alternative splicing is a significant contributor to transcriptome diversity, and a high-throughput experimental method to assess quantitatively predictions from EST and microarray analyses may help to answer questions about the extent and functional significance of these variants. Here we describe a method for high-throughput analysis of known or suspected alternative splicing variants (ASVs) using PCR, primer extension and MALDI-TOF mass spectrometry. Reverse transcribed mRNA is PCR amplified with primers surrounding the site of alternative splicing, followed by a primer extension reaction designed to target sequence disparities between two or more variants. These primer extension products are assayed on a MALDI-TOF mass spectrometer and analyzed automatically. This method is high-throughput, highly accurate and reproducible allowing for verification of the existence of splicing variants in a variety of samples. An example given also demonstrates how this method can eliminate potential pitfalls from ordinary gel electrophoretic analysis of splicing variants where heteroduplexes formed from different variants can produce erroneous results. The new method can be used to create alternative variant profiles for cancer markers, to study complex splicing regulation, or to screen potential splicing therapies.

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Comprehensive Assessment of Metabolic Enzyme and Transporter Polymorphisms. *D. Fu[1], T. Daly[1], P. Hardenbol[2], X. Miao[2], N. Bauer[1], S. Kirkwood[1], R. Hockett[1].* 1) Genomic Medicine Group, Diagnostic and Experimental Medicine, Eli Lilly & Co, Indianapolis, IN; 2) ParAllele Biosciences, South San Francisco, CA.

Understanding drug metabolism and transport are increasingly important to the concept of personalized medicine, accounting for a significant fraction of therapeutic unresponsiveness and/or adverse events. Currently available assay systems for genotyping patients measure only a fraction of the known drug metabolic enzymes and transporters (DMETs). We developed a genotyping platform to comprehensively measure ~2,000 polymorphisms in the 184 known DMETs. The system uses ParAlleles Molecular Inversion Probes and Affymetrix Tag arrays. A subset of 29 genes (171 variants), with documented effects on drug disposition, have been validated for clinical use according to regulatory guidelines. Since the field is rapidly changing, with the discovery of new genes and new variants, the sytem was designed to be flexible, robust, and cost effective. The utility of a comprehensive assessment of DMETs for drug development revolves around building a patient database of know genotypes that could be recruited into new clinical trials. A thorough understanding of DMETs will also aid the investigation of drug related adverse events and the exploration of proper drug dosing. A review of 750 patients, on all 2,000 variants, will be presented, with particular emphasis on the frequency of rare alleles.

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Detailed Genotype Comparison of ABI Taqman, SNPlex and Illumina Platforms. *J. Rose, J. Bunch, C. Haynes, E.R. Hauser, M. Pericak-Vance, J.M. Vance, S.G. Gregory.* Department of Medicine, Duke Center for Human Genetics, Durham, NC.

The increasing application of SNP-based linkage and association analyses to the study of disease research has been driven by the development of genotyping platforms that are capable of multiplexing assays. Our aim was to compare the pass and call rates of the Illumina Bead Array and ABI SNPlex platforms with ABI Taqman using 460 samples derived from an on going coronary artery disease study. A total of 19 SNPs were genotyped on all three platforms across 460 DNAs, with a further 1119 DNAs being in common between SNPlex and Taqman. Our controls consisted of two in-plate QC samples and two standard across-plate CEPH samples for Illumina and SNPlex, and 12 in-plate/across-plate QC samples and two standard across-plate CEPH samples for Taqman. The quality of 41 samples that had been whole-genome amplified by Molecular Staging Inc., as reported by the manufacturer, showed no effects on Taqman pass rates. In both Illumina and SNPlex platforms, a similar trend was observed with a decreasing pass rate associated with 3 useable amplified samples (75% average pass rate) and 2 not useable amplified samples (average 52% pass rate). Of the 384 SNPs included in the custom Illumina Oligo Pooled Assay, 22 were excluded due to poor performance. The in-plate controls for Illumina showed only 6 mismatches when compared to Taqman results out of a possible 3840 matches, which dropped to zero after the exclusion of 22 poor markers. The sample match rate to Taqman was also perfect after exclusion of these 22 samples. The remaining 362 Illumina markers had an average call rate of 99.3% (high=100%, low=92.5%). Of the 47 SNPs used in the SNPlex assay, 4 were excluded due to poor performance (<55% call rate). The remaining 43 SNPlex markers had an average call rate of 88.15% (high=98.49%, low=55.94%), with an average mismatch rate compared to Taqman of 1.12% (high=5.08%, low=0.21%). Together, these data suggest that Taqman and Illumina results are very reliable after stringent exclusion of poorly performing markers, however, SNPlex genotyping resulted in genotyping discrepancies and lower efficiencies.

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Academic three-color microarrays passing prehybridization QC generate data quality equivalent to that of commercial array systems. *M. Hessner[1,2], B. Xiang[1], R. Geoffrey[2], S. Holmes[1], S. Jia[1], L. Meyer[2], X. Wang[1,2].* 1) Dept Pediatrics, Medical College of WI, Milwaukee, WI; 2) The Human and Molecular Genetics Center, Medical College of WI, Milwaukee, WI.

Despite their lower cost and high content flexibility, a substantial limitation of academically fabricated arrays is their susceptibility to quality control (QC) issues. To solve this we developed a novel three-color system where arrays are directly visualized prior to hybridization, allowing for QC of slide fabrication independent of hybridization. We benchmarked our rat cDNA array against two common commercial systems, the Affymetrix U34A and Agilent G4130A arrays, utilizing liver RNA of Wistar and BioBreeding rats. 2,824 Unigenes represented on all three arrays served as the comparison basis. First, we identified probes that yielded significant t-test results ($p<0.05$) and passed their respective quality control criteria using the 50% cutoff established by the default Affymetrix MAS 5.0 thresholds. Probes for 79 UniGenes met these criteria and Pearsons correlation coefficients of 0.95, 0.92, and 0.91 were determined for the overall $\log_2$ ratios between the Affymetrix U34A versus the Agilent G4130A, the Affymetrix U34A versus the MCW cDNA array, and the Agilent G4130A versus the MCW cDNA array, respectively. To date, we have conducted real-time qRT-PCR for 42/79 of the common loci that were significantly detected on all three platforms. All platforms showed good agreement with qRT-PCR, possessing Pearsons correlation coefficients of 0.90, 0.90, and 0.96 for the Affymetrix, Agilent, and MCW cDNA arrays, respectively, however each platform underestimated $\log_2$ ratios relative to qRT-PCR, possessing respective slopes of 0.86 (R^2=0.81), 0.65 (R^2=0.81), and 0.70 (R^2=0.92). We find differentially expressed genes among the highest quality data derived from each platform are highly correlative. Our combined novel three-color array and unique QC/analytical approaches provide a robust platform for functional genomics that rivals commercially available systems at approximately 1/10th the cost, allowing for experimental designs less constrained by cost considerations.

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Computer simulations of the Genomic State Reconstruction dynamical systems algorithm for within-individual predictive systems biology. *I.P. Kovac, A.J.M. Sorant, A.F. Wilson.* Genometrics, NHGRI, Baltimore, MD.

The Genomic State Reconstruction (GSR) is a dynamical systems algorithm for bottom-up within-individual prediction and delineation of genomic basis in complex biological systems, such as common human systems-level disorders (Kovac, unpublished manuscript, etc; McGill University). Briefly, the state (phenotype) of the dynamical system (individual organism) is predicted using the high-dimensional reconstructing vector of one-to-one genomic or genomic-subset similarity measurements (by the proportion of identical allelic states) between the specific within-individual polygenic allelic combination and a plurality of affected individuals, based on the prevailing assumption of positive correlation between genomic and phenotypic similarity. Comprehensive exposition of the GSR algorithm is presented elsewhere. The subsequent simulation study now includes up to 20 SNP loci, population of 5000 individuals, and phenotype prevalences of .01 and .02. Polygenic phenotypes included within-individual loci-additive, loci-multiplicative, and emergent systems-level phenotypes where units of phenotypic information are developmental systems combinations of different loci. The GSR successfully predicted all phenotypes, without specification of the underlying polygenic model. Using the complete set of etiological loci, individuals ranged from those well below population risk to those at elevated risk, up to several ten-folds or more over population risk. The truncation of etiological loci and/or addition of non-etiological loci typically decreased predictive accuracy, the more so with the number of truncated or added loci. For emergent phenotypes the amount of decrease due to truncation depended on systems relations. These predictive differences were used to delineate maximally predictive within-individual genomic subset. As mathematically expected, the GSR performed well only when the noted prevailing assumption was built into the models. The results apply to other population and data sizes. Computer simulations provide additional support for experimental research to empirically evaluate the GSR algorithm.

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SNP Genotyping: DNA quantification with the PicoGreen assay and clustering of SNP genotypes utilizing the Illumina GoldenGate assay and BeadArray technology for varying DNA concentrations. *K. Hetrick, C. Bark, Y. Tsai, C. Boehm, E. Pugh, K. Doheny.* Center for Inherited Disease Research, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Illumina utilizes a single read of the PicoGreen assay (pico) to evaluate input genomic DNA concentration for the SNP GoldenGate assay (50 ng/ul minimum). Investigators often only have OD 260 readings available and limited sample DNA. We examined single-point pico readings and clustering of genotypes for a range of concentrations in order to establish a practical cut-off for rejecting a sample for processing. A single CEPH DNA sample (356 ng/ul by OD as provided by the Coriell Cell Repository) was serially diluted into 11 aliquots of different concentrations. Pico readings were performed via the Illumina BeadLab Make QDNA protocol (dynamic range 0-100ng/ul for input DNA) on two different robots. These samples were genotyped and clustered by the Illumina BeadLab GenCall (Version 6.0.7) software after processing the samples through the GoldenGate assay. For concentrations between 10-100 ng/ul, the mean pico readings were slightly lower than expected by OD with standard deviations (s.d.) of ~10% of the pico means. The mean for water was 0.39 with a s.d. of 0.48. Pico reading for samples beyond the dynamic range of assay increased only slightly with concentration. Genotypes for water samples did not cluster with the other genotypes. Genotypes for samples at 10, 25 and 356 ng/ul were more likely to be greater than 1 s.d. from the cluster mean of a locus. Because all genotypes came from one individual and only the best 874 loci of an 1152 SNP panel were used for analysis, these results may be atypical. Based on our results, we are currently rejecting samples with a pico reading of less than 25ng/ul. We are currently comparing clustering for investigator provided samples of varying DNA concentration.

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An Automatic Sequencing-based Method of Genotyping for Tri-nucleotide Polymorphisms as a Replacement / Complementary Approach to Fragment Analysis. *S. Xu, E. Wang.* Department of Biochemistry and Molecular Biology and Gheens Center on Aging University of Louisville School of Medicine 580 South Preston Street Delia Baxter Building, Room 109 Louisville, Kentucky 40292.

Trinucleotide repeats are among the most polymorphic markers utilized in linkage and association studies. Up till now, genotyping of these repeats has been done either by agarose or polyarylamide gel electrophoresis, or by capillary electrophoresis coupled with the automatic DNA sequencing analysis. In either cases quantitation of the triplets is obtained by inference of electrophoretic mobility by comparing them with a standard, thus an indirect method in calculation of the repeats. Here, we report a new method to obtain the direct measurements of the triplet repeats, thus allowing accurate genotyping for three triplet repeat loci: androgen receptor (AR), myotonic dystrophy (DM, and Huntingtons disease (HD). This direct measurements is done by designing the primers flanking the triplet repeat regions in the way that the outcome genotype in sequencing format be analysed by counting the number of A or T peaks alone, contained in whole triplet tracts. Specific starting and ending motifs defined by properly designed primers, as well as graphics visualization by means of a single sequencing reaction, make it possible to read out homozygous as well as heterozygous genotypes, not only with ease but also without ambiguity. In addition, this method avoids the need of loading DNA ladders and thus is cost- and time-effective; suitable for both small and large-scale genotyping. Moreover, the genotyping data generated by our method can be analyzed by any commercially available image processing softwares and provide value-added high-throughput power for the final datamining tasks. With this approach we did genotyping and obtained allele frequencies of AR, DM and HD in Taiwanese population accurately.

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Performance Analysis and Improved Base-Calling for Large Scale Resequencing Arrays. *B. Merriman[1], M. Ogdie[1], M. Kane[2], J. Jen[2], Z. Chen[1], S. Nelson[1].* 1) Dept. of Human Genetics, University of California, Los Angeles, Los Angeles, CA; 2) Dept. of Neurology, University of California, Los Angeles, Los Angeles, CA.

Hybridization-based resequencing on high density DNA oligo arrays provides a potentially attractive approach to large scale resequencing projects, such as screening large numbers of samples and genes for rare variants related to a disease. Here we present a detailed analysis of the performance of an 30kb resequencing array (from Affymetrix) designed to resequence the coding portions of 4 human genes, comprising 28800 bases of sequence from chromosome 3. We find a very high proportion of the probes (~98%) give signals consistent with the consensus sequence, and that probes also exhibit several consistent indicators for detection of non-consensus sequence. We also show that there are a variety of hybridization artifacts that systematically affect the probe performance, and in particular impact accurate base detection, and that these artifacts can be reliably identified largely from the local sequence context. Based on these fundamental performance observations, we present a new base calling algorithm which substantially improves upon the call rate and heterozygous detection capabilities of the standard software (Affymetrix GDAS 3.0), as validated by capillary sequencing and mendelian inheritance analysis in familial sample sets. Thus array-based resequencing can be a reliable means of resequencing for disease variant detection. The sequence-based artifacts identified also suggest directions for future development of improved array designs or protocols, relevant to general array formats. Finally, as a novel application of the technology, we show that the human *re*-sequencing arrays can be used for the *de-novo* sequencing of primate species. We test a variety of primate samples, show they have very similar hybridization properties to the human samples, and, using our enhanced base-calling algorithms, we clearly identify numerous instances of non-conservative human-primate sequence differences.

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Novel High-throughput Assays using Shape Encoded Particles. *Z. Chen[1], B. Merriman[1], J. Tsai[2], M. Xie[1], J. Chen[1], C.J. Kim[2], S. Nelson[1].* 1) Dept. of Human Genetics, University of California, Los Angeles, Los Angeles, CA; 2) Micromanufacturing Laboratory, Dept. of Mechanical and Aerospace Engineering, University of California, Los Angeles, Los Angeles, CA.

We have developed a novel system for highly multiplexed biological assays using shape encoded micro-particles as probe-carriers. Millions of distinct Shape Encoded Particles (SEPs) can be created by etching unique patterns of notches on the edge of flat silicon particles using standard microfabrication techniques. The system has been demonstrated as probe carriers for oligonucleotide and protein binding assays, where it provides an attractive alternative to microarray-based techniques due to the potential for efficient mass production, high through-put, scalability and flexibility. Here we extend this basic technology to allow multiplexing of much more general types of biochemical assays. First, we have extended this approach to cellular assays, using the SEPs as encoded cell carriers. As a demonstration, different cancer cell lines were grown on distinct shapes, mixed, and induced for programmed cell death. Cell-line specific apoptosis was clearly detectable on SEPs, illustrating the possibility of multiplex cell-based drug or functional screening. Second, we extended the SEP approach to multiplex complex reactions that normally require individual tubes or wells, by making Shape Encoded Micro-Reactors (SEMRs), consisting of particles with a micro-well (1 picoliter1 nanoliter volume) etched into the center. Using novel techniques for parallel loading and sealing, a standard assay for proteinase activity was successfully multiplexed into the SEMR system, to create the equivalent of an enzyme array capable of screening activities of many enzymes in one macro-scale reaction. This has potential application to large-scale enzyme inhibitor screening, and also illustrates the utility of SEMRs for multiplexing arbitrary biochemical reactions.

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Increased Efficiency of SNP Genotyping on Sequenom MassArray. *L. Ziaugra[1], N. Burtt[1], I. Musil[1], M. Goyette[1], T. Considine[1], M. Beaullieu[2], D. Lough[2], E. Munk[2], D. Altshuler[3], S.B. Gabriel[1].* 1) Broad Inst, Cambridge, MA; 2) Sequenom INC., San Diego; 3) Harvard Medical School and Massachusetts General Hospital.

Several high throughput genotyping platforms now offer multiplexing of SNPs at the level of thousands of SNPs at once. While these technologies are enabling for whole genome scans, multiplexing at a more modest level will be required to quickly and efficiently follow up results of these large scale association studies. The iPLEX technology is a newly developed SNP genotyping assay for use on Sequenom MassArray platform. The most significant difference of the iPLEX compared to the existing Sequenom MassArray assay(hME) is that all reactions are terminated after a single base extension (SBE). The other important feature is that iPLEX assay incorporates mass-modified di-deoxyterminators thus allowing better mass resolution (15Da) and a four fold increase in multiplexing capacity (20-30 plex for iPLEX compared to 5-7 plex with hME). We performed a pilot study of 97 SNPs which were designed into five multiplex pools (4-23 plex and 1-5plex). The pools were genotyped on the HapMap CEU plate. Results obtained with the iPLEX assay were compared to genotype calls made using the previously well-validated Sequenom hME assays for the same SNPs. Of the 97 SNPs, 80 passed genotype quality metrics yielding 99.7% concordance with consensus genotypes and averaged 97% call rates. The SNP pools will be applied to a large case control study.

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Selecting tag SNPs of 328 candidate genes responsible for adverse effects of cancer chemotherapeutics. *M. Isomura[1], H. Fujisawa[2], M. Ushijima[1], S. Miyata[1], M. Matuura[1], Y. Miki[1].* 1) Genome Center, Cancer Institute, Tokyo, Japan; 2) Institute of Statistical Mathematics, Tokyo, Japan.

Appearance of adverse effects of cancer chemotherapeutics depend on polymorphisms of recipient'fs genome. As demonstrated on certain chemotherapeutics, deleterious mutation on genes related with drug metabolism increase risk of adverse effects. However, frequencies of those mutations are relatively low. Thus, those are insufficient to predict most of severe adverse effects that may cause interruption of treatment, although those polymorphisms are useful to prevent lethal event on a small number of patient. A number of low risk polymorphisms have to be identified to construct prediction system of adverse effects on chemotherapeutics. To screen polymorphisms associated with adverse effects of chemotherapeutics more efficiently, we attempted to estimate haplotype block structure of candidate genes to select tag SNPs. To elucidate genes responsible for adverse effects of chemotherapeutics, we selected 507 candidate genes. These included genes related to drug metabolism, DNA repair, apoptosis, cell cycle regulation, angiogenesis, and inflammation. SNPs located in or around those genes were obtained from JSNP databases, and 3,246 SNPs were selected for genotyping. Genotypes of those genes were determined on 1,421 cancer patients by Invader assay. SNPs with low minor allele frequency (0.1), and those departed from Hardy-Weinberg equilibrium were eliminated from further analysis. In addition, genes covered by only one SNP were eliminated. By those selections, 2,249 SNPs covering 328 genes were used for haplotype block analysis. After estimating haplotype frequencies of genomic loci of those genes, haplotype block structures were estimated by ADBlock software, which is based on ancestor derived model and MDL principle. Those analyses revealed that genomic loci of 328 genes were subdivided into 547 haplotype blocks. The average length of haplotype block was 18.7kb and the average number of major haplotypes in each haplotype block was 3,7. Based on haplotype block structure, 1,432 tag SNPs, representing more than 90 % of haplotypes observed in 547 haplotype blocks, were selected.

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Gene Entrapment Libraries For mouse Functional Genomics. *Q. Lin, S.L. Donahue, T. Moore-Jarrett, S. Cao, A. Nashabi, A. Osipovich, H.E. Ruley.* Microbiology & Immunology, Vanderbilt University, Nashville, TN.

Mutations induced by gene entrapment in murine embryonic stem (ES) cells have been widely used to characterize gene functions in mice. However, entrapment mutations are typically recessive and can be studied only after they have been introduced into the mouse germline and bred to a homozygous state. The present study describes novel poly(A) gene trap vectors capable of targeting both alleles of mammalian genes with a single gene entrapment event. The vectors, which contain features to facilitate the identification of disrupted genes and for post-entrapment genome engineering, were used to characterize a library of 980 mutant stem cells. All 10 mutations tested were easily transmitted into the germline and 4 of them generated obvious phenotypes, establishing the library as a resource for constructing mutant mice. For example, Hesx1 -/- mice have an ¡eyeless¡ phenotype, pfdn1 (Prefoldin) -/- mice die before 5 weeks of age, have CNS abnormalities consistent axon extension defects and deficiencies in B-cell development. A neomycin resistance gene (Neo) inserted by the vector was used to select for homozygous mutant cells in 36 of 37 entrapment clones following selection for higher levels of drug resistance. The frequency of apparent LOH increased with increasing distance from the centromere, suggesting that mitotic recombination plays a significant role in generating losses of heterozygosity. The ease and efficiency of obtaining homozygous entrapment mutations will enhance the utility of mutant stem cell libraries by eliminating the need to pass mutations through the germline in cases where functional studies would be best performed in cell lines rather than in mice. Moreover, the vectors will enable new strategies to identify genes responsible for recessive phenotypes in mammalian cells.

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Array-CGH deletion mapping excludes the involvement of VCXA in mental retardation. *E. Scala[1], C. Pescucci[1], R. Caselli[1], I. Meloni[1], D. Lugtenberg[2], F. Mari[1], H. van Bokhoven[2], A. Renieri[1].* 1) Molecular Biology, University of Siena,Italy; 2) Dept of Human Genetics, University Medical Center of Nijmegen, The Netherlands.

VCXA has been recently proposed as a candidate mental retardation (MR) gene. To better define the involvement of this gene in MR we have performed array-CGH deletion mapping using a 200 kb resolution X chromosome specific BAC arrays. We have compared the deletion present in a familial case with X-linked ichthyosis (XLI) and MR to that present in a sporadic case with XLI and Kallmann syndrome (KAL). In the case with XLI and KAL, array-CGH analysis showed the presence of a large deletion of approximately 3 Mb. The deletion includes the STS gene, the KAL1 gene (responsible for KAL) and also VCXA, VCXB, VCXB1 and VCXC genes. In the case with XLI and MR the analysis revealed the presence of a smaller deletion, approximately 0.6 Mb, spanning from clone RP11-483M24 to clone RP11-645P06. The deletion does not include the VCXA gene, which has been previously suggested to be involved in mental retardation. The deletion involves the STS gene responsible for XLI (OMIM#308100) and only a second gene, named HDHD1A. This gene, also known as GS1 (OMIM#306480), has been reported deleted in patients with isolated XLI. The deletion map in these two cases exclude the involvement of the VCX genes in mental retardation. A possible involvement of GS1 as a low penetrance MR gene is discussed.

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NCBI Entrez Phenotype Resources. *M.D. Mailman, S.T. Sherry, D.R. Maglott.* National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Dr., Bethesda, MD, 20892, USA.

NCBI recently launched a new Entrez database, PhenoDB, to represent phenotypes, possible causative factors, and the defining assays. In the data model, potential contributing factors include genotype (ie: haplotypes, SNPs, alleles), epigenetic status, gene expression, exposure to drugs or atmospheric conditions, diet, previous occurrence, and ancestry. A phenotype is linked to a controlled vocabulary or ontology term when possible, such as the Mouse Genome Informatics (MGI) Mammalian Phenotype Ontology or Gene Ontology, and has an associated species (NCBI Taxonomy), gender, relevant anatomical site (Foundational Model of Anatomy and species-specific vocabularies), species-specific developmental stage, inheritance pattern, and penetrance. Phenotypes can be associated with one or more diseases from the MeSH vocabulary, the NCI Metathesaursus, the Online Mendelian Inheritance in Man (OMIM), or the Online Mendelian Inheritance in Animals (OMIA). We have linked over 1000 SNPs from dbSNP to allelic variants described by OMIM and have linked diseases in OMIM to phenotypes by mapping OMIM Clinical Synopses to the Mammalian Phenotype Ontology. A similar mapping has been made between phenes in OMIA and the Mammalian Phenotype Ontology. In addition, PhenoDB models the raw data and the assays that are used to generate them. This is important because phenotypic data is relevant only in the context in which it was measured. PhenoDB will store phenotype and phenotype-related data from any species. Cross-species comparisons will be facilitated by linkage to common phenotype ontology terms. We expect that this resource will be useful in the generation of hypotheses about factors that could be associated with a phenotype using the power of our knowledge of factors in multiple species. Integration of PhenoDB with the NCBI Entrez system allows for convenient linkage to relevant records in other databases at NCBI such as dbSNP, Gene, GEO, Taxonomy, OMIM, OMIA, Probe, MapViewer, and PubMed. Links will also be provided, directly or indirectly, to data contributors or ontology providers such as MGI, Rat Genome Database, PhysGen, NCI, MeSH, and Gene Ontology.

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Clinically relevant VKORC1 haplotype and CYP2C9 genotype frequencies in multiple world populations. *S. Marsh, C.R. King, R.M. Porche-Sorbet, T.J. Scott-Horton, C.S. Eby, B.F. Gage, H.L. McLeod.* Washington University School of Medicine, St Louis, MO.

Warfarin, a commonly used treatment for preventing blood clotting, has a narrow therapeutic index, and over or under-dosing can be life-threatening. Warfarin is metabolized by CYP2C9 and the vitamin K epoxide reductase complex (VKORC1) is the drug target. Recent studies have identified haplotype-dependent predictions for warfarin dosing. In addition, CYP2C9 genotype has also been described as a predictor for warfarin dose. VKORC1 haplotype predicted 21-25% of the required warfarin dose, and CYP2C9 genotypes reduced the required warfarin dose up to 31% in Caucasians. Screening of VKORC1 and CYP2C9 prior to initiating therapy will provide essential information for improving dose selection. Population differences of VKORC1 haplotype and CYP2C9 genotype frequencies have also been observed. In order to identify the high risk genotype combination frequency across world populations we assessed four haplotype tag SNPs for VKORC1 (685, 861, 5808 and 9041) and CYP2C9 (*2 and *3) using PCR and Pyrosequencing in 556 unrelated healthy individuals from European American (95), African (95), African American (95), Hispanic (Mexican (95) and Peruvian (81)) and Asian (95) populations. VKORC1 haplotypes were classified into Haplotype A (low warfarin dose) and Haplotype B (high warfarin dose) groups. Haplotype A group frequencies ranged from 27% in Peruvians to 85% in Asians. Caucasians had a Haplotype A group frequency of 42%. CYP2C9 polymorphisms were more frequent in Caucasians (14% CYP2C9*2, 6% CYP2C9*3) and were least common in Peruvians (1% for both variants).Caucasian populations had the highest frequency of combined VKORC1 and CYP2C9 polymorphisms (18% of individuals had at least one VKORC1 haplotype A and at least 1 CYP2C9 variant allele). Based on VKORC1 haplotype frequencies and CYP2C9 polymorphisms, these data suggest that Caucasian and Asian patients have higher risks of warfarin overdose whilst African and Peruvian populations are likely to have greater warfarin-dose requirements.

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NIEHS Environmental Genome Project: Functional Significance of Environmentally-Responsive Genotypes for Human Disease. *K. McAllister, F. Tyson, L. Maull, K. Gray, S. Srinivasan, L. Reinlib, G. Collman.* DERT, NIEHS, Res Triangle Park, NC.

The National Institute of Environmental Health Sciences initiated the Environmental Genome Project (EGP) to identify and characterize genetic variants that influence human susceptibility to environmental agents. The EGP is a multi-component project comprised of research in the following four areas: (1) The resequencing effort initially focused on a panel of 90 human samples to identify single nucleotide polymorphisms (SNPs) in ERGs and a new panel of ethnic identifiers has recently been added. This effort now includes 466 ERGs related to DNA repair, cell cycle control, cell signaling, cell division, homeostasis, and metabolism. The GeneSNPs database, http://www.genome.utah.edu/genesnps/, integrates this gene, sequence, and polymorphism data into individually annotated gene models. (2) The Comparative Mouse Genomics Centers Consortium has approximately 60 SNP and tool mice strains developed from DNA repair and cell cycle genes that display aging, diabetes, and cancer phenotypes. Validated mouse models and resources developed by the CMGCC are available to the scientific community and can be accessed at the CMGCC website, http://www.niehs.nih.gov/cmgcc. (3) The molecular epidemiology planning grants have provided insights into the environmental susceptibility of several neurodegenerative diseases, childhood leukemia, birth defects, and asthma. (4) Finally, the ELSI grants have focused on developing policies to effectively deal with the concerns of the community relating to environmental health research. Insights into the functional relevance of the identified human DNA polymorphisms from these projects will contribute to a greater understanding of the complex relationships between genetic susceptibility, environmental exposures, and human disease.

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Mutation Screening of WDR36 and Two of Its Binding Partners, MAX and E2F6, in Families with Adult-Onset Primary Open Angle Glaucoma (POAG). *S. Monemi[1], G. Spaeth[2], E. Ilitchev[3], J. Liebmann[3], R. Ritch[3], E. Heon[4], R.P. Crick[5], A. Child[6], M. Sarfarazi[1].* 1) Dept Surgery, Univ Connecticut Health Ctr, Farmington, CT; 2) Wills Eye Hospital, Philadelphia, PA; 3) Glaucoma Service, New York Eye and Ear Infirmary, NY; 4) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Canada; 5) International Glaucoma Association, London, UK; 6) St. Georges Hospital Medical School, London, UK.

Recently, we mapped a new locus *(GLC1G)* for Adult-Onset POAG to 5q22.1 and subsequently identified *WDR36* as a novel causative gene at this locus. Screening of this gene in 130 unrelated POAG families revealed 4 disease-causing mutations (N355S, A449T, R529Q and D658G) and 20 other DNA variations. These evolutionary conserved mutations observed in a total of 17 unrelated subjects, 11 with high- and 6 with low-pressure glaucoma. By Northern blotting and RT-PCR, expression of this gene and its homologue, *Wdr36*, were established in human and in mouse, respectively. The mRNA expression profiling of *WDR36* in different human ocular tissues was consistent with its role in the etiology of glaucoma. WDR36 is a 951 a.a. multi-tasking protein with different motifs (i.e., G-protein beta WD40 repeats). As this protein may function in a number of biological processes and/or participate in different biochemical pathways, identification of WDR36-interacting proteins and their encoding genes may provide new insights for delineation and underlying molecular mechanisms of glaucoma. Recently, transcription factor E2F6 and MAX proteins were reported as WDR36-binding partners. Therefore, we aimed to screen their encoding genes in a group of unrelated glaucoma families. Haplotype analyses of a group of 139 POAG families recently scanned for the entire genome revealed potential genetic linkages to 2p25.1 and 14q23 regions, encompassing *E2F6* and *MAX* genes, respectively. Direct DNA sequencing of *MAX* in a group of 10 POAG families revealed only 7 intronic variations, including 3 novel SNPs. DNA sequencing of *E2F6* in another group of families is currently in progress. Supported by EY-009947 and M01RR-06192.

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Exceptionally low LD and high recombination in human immune genes. *X. Ke[1], J. Kelley[2], G. Abecasis[3], J. Marchini[4], R. Copley[1], J. Trowsdale[2], L.R. Cardon[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Pathology, University of Cambridge, UK; 3) Center for Statistical Genetics, Dept. of Biostatistics,University of Michigan, USA; 4) Department of Statistics, University of Oxford, UK.

The human genome is estimated to contain 20,000-25,000 protein coding genes, including about 20,000 known genes. Of these known genes, at least 7% are estimated to be involved in the human immune system. Many immune genes, particularly those in the major histocompatibility complex (MHC), have been studied intensively. The profile of linkage disequilibrium (LD) and historical recombination events of the immune genome, however, is still not well understood. In the present study, we have carried out a genome-wide survey of the level of LD, LD decay, and recombination rates on immune genes in CEPH, Chinese, Japanese, and Yoruban populations using genotype data from the International HapMap Project. LD of immune genes is significantly lower than LD of genes in the rest of the human genome. Immune genes are also associated with quicker LD decay and higher recombination rate. Among the immune genes, those interacting directly with pathogens usually have less LD and higher recombination rate, whereas those processing antigens have the highest LD and least recombination. The antigen-processing genes, such as are commonly found in the MHC, are also found to have marginally stronger LD than the genome average. These observations reflect the complexity of the human immune system as well as the evolution and conservation of the individual immune genes.

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Microarray gene expression profiling of skin melanocytes from patients with vitiligo. *C.M. Mailloux[1], G.D. Shipley[2], R.A. Spritz[1].* 1) Human Medical Genetics Program, University of Colorado Health Sciences Center-Fitzsimons, Aurora, CO; 2) Cascade Biologics, Inc., Portland, OR.

Generalized vitiligo is a common polygenic, multifactorial disorder in which white patches of skin and hair result from autoimmune loss of pigment-producing melanocytes in these areas. Vitiligo is highly associated with other autoimmune diseases, and appears to involve a non-inflammatory T-cell immune response directed against pigment cells. Nevertheless, relatively little attention has been paid to cell-autonomous properties of the cellular target, the melanocyte. To address this, we have carried out genomewide expression profiling analysis of vitiligo melanocytes. Although it is extremely difficult to grow primary skin melanocytes from adults, we cultured melanocytes from uninvolved skin of two affected sisters from a large family in which vitiligo has been linked to chromosome 1p, their unaffected mother, and a number of unrelated female controls. We extracted RNA from the cultured melanocytes and carried out genomewide RNA expression profiling using Affymetrix HG-U133 plus 2.0 microarrays, which interrogate 47,000 transcripts. Data from replicate samples showed very high correlation, and cluster analysis grouped the affected patients separately from the controls. Gene expression profiles of the affected patients were compared to profiles of the controls, and genelists of significant expression changes, ranked by fold-differences and by *p*-values of the differences, were compiled. The data were subjected to pathway analysis to organize genes into functional groups, and genes were sorted by chromosomal location for comparison with genetic linkage data for vitiligo. These analyses identified a limited set of genes whose expression was significantly up- or down-regulated, most notably including an HLA gene and a number of genes involved in regulating melanocyte cell-cycle and cell division. Our studies thus suggest there may be primary dysregulation of melanocyte growth in at least some patients with vitiligo, and identify potentially important candidate genes that may contribute to the pathophysiology of vitiligo and other associated autoimmune diseases.

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Serum biomarker discovery for MPS disease: Utility of the murine MPS I model. *D.R. Randall[1], G.B. Sinclair[2], K.E. Colobong[1], L.A. Clarke[1].* 1) BC Research Institute for Childrens and Womens Health, Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Biochemical Genetics Laboratory, Department of Pathology and Laboratory Medicine, Childrens and Womens Health Centre of BC, Vancouver, BC, Canada.

Objective: Mucopolysaccharidosis I (MPS I) results from an inherited deficiency of the lysosomal enzyme -L-iduronidase (IDUA), causing an altered ability of cells to metabolise heparan and dermatan sulphate containing glycosaminoglycans (GAGs). These GAGs accumulate in the lysosomes of virtually all cells and subsequently lead to cellular, tissue, and multi-system organ dysfunction. Currently, there are few objective criteria available to evaluate the burden of disease in MPS I nor responsiveness to therapeutics. The serum proteome presents a potential source of protein biomarkers for MPS I management but is technically challenging due to the presence of high-abundance proteins that mask detection of useful proteins. **Methods:** Two independent pools of serum from six 20-week-old IDUA knock-out mice and matching control samples were depleted of albumin, transferrin, and IgG. Aliquots were analysed by isotope tagged (iTRAQ) differential proteomics and by 2-D gel electrophoresis (2DGE) with and without prior solution-phase isoelectric focusing (IEF). **Results:** 2DGE revealed multiple proteins with altered expression levels representing possible biomarkers for MPS disease. IEF prefractionation will be used to increase sample loading for protein identification and relative quantitation of low abundance species. iTRAQ was used to quantify and compare the expression of more than 200 proteins and indicated a homeostatic compensation mechanism of factors involved in coagulation to resist the burden of disease imposed by GAG accumulation. Such factors may be ideal biomarkers to enhance clinical management of MPS patients and MPS research. **Conclusion:** Prefractionation methods are effective tools in probing the serum proteome for candidate biomarkers of disease. We have identified several potential biomarkers for MPS I disease utilizing the mouse model; further validation of these markers in humans is now underway.

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Assessment of schizophrenia genomes for DNA copy number alterations. *S.E. Holmes[1], N.A. Sachs[1], J.K. Cowell[2], J. Conroy[2], N.J. Nowak[2], D. McQuaid[2], M.R. Rossi[2], D.P. Gaile[2], S.L. Christian[3], M. Wigler[4], J. Sebat[4], C.A. Ross[1], L.E. DeLisi[5], R.L. Margolis[1].* 1) Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD; 2) Roswell Park Cancer Institute, Department of Cancer Genetics, Buffalo, NY; 3) Department of Psychiatry, The University of Chicago, IL; 4) Cold Spring Harbor Laboratory of Biology, Cold Spring, NY; 5) Department of Psychiatry, New York University, New York, NY.

Introduction: Comparative genome hybridization array (aCGH) and Representational Oligonucleotide Microarray Analysis (ROMA) were used to determine whether genomes of individuals with schizophrenia contain deletions and/or duplications. Methods: Polymerase chain reaction representations of 6,116 bacterial artificial chromosomes (BACs) spanning the human genome at 0.5-1 mb intervals were arrayed in triplicate for aCGH, while approximately 85,000 70-mer oligos with an approximate 100 kb resolution were used for ROMA. Subject genomic DNA was derived from lymphoblastoid cell lines of 27 individuals with schizophrenia and 6 individuals with schizoaffective disorder, all from families with multiple members affected with schizophrenia or other major mental illness originally ascertained as part of a genetic study of sibpairs. In each array, uniquely labeled subject and control DNA is hybridized and alterations in hybridization may correspond to DNA copy number changes. Additionally, aCGH analysis of genomic DNA isolated from control lymphoblastoid cell lines was hybridized against stock control DNA to determine the baseline rate of abnormal hybridization signals. Altered hybridization signals from subject DNA at an equivalent BAC to that observed in control DNA were generally disregarded in future analyses. Results: Real-time qPCR assays were designed to test the 27 most significant regions of DNA copy number changes detected by aCGH or ROMA. Thus far, a deletion detected by aCGH at 5p15.1 in a subject with schizophrenia has been substantiated by qPCR. Conclusions: Two previous linkage findings are close to 5p15.1, supporting the notion that deletion of this region may contribute to the genetic risk of schizophrenia.

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Identification of disease causing mutations using high-density whole-genome SNP genotyping microarrays. *D.W. Craig[1], M.J. Huentelman[1], D. Hu-Lince[1], K.D. Coon[1], K.M. Ramsey[1], J.P. Parod[1], M.C. Kruer[1], J.M. Pearson[1], E.G. Stephan[2], D.A. Stephan[1].* 1) Neurogenomics Division, TGen, The Translational Genomics Division, Phoenix, AZ; 2) Clinic for Special Children, Lancaster, PA.

The technology to simultaneously genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) in a single assay has only recently been developed. These technological SNP genotyping advances have the potential to revolutionize our ability to identify disease-associated genes, both in complex and in Mendelian inherited diseases. We describe application of this technology for discovery of novel mutations using several different experimental approaches. First, for familial linkage studies we describe using the Affymetrix GeneChip 10K SNP Mapping Arrays to discover disease-causing mutations for four separate disorders. Second, we describe using copy number analysis of SNP genotyping microarrays as an approach to discover a novel amplification and a novel deletion in two separate disorders. Third, we describe approaches for pooling genomic DNA on the 100K and 500K SNP Mapping Array platforms as a pre-screening approach in association studies of complex disorders. Finally, we describe our progress towards genotyping 7,000 individuals for 10,205 SNPs as part of the Autism Genome Project and our progress towards completion of a 2000 case-control whole-genome association study in Alzheimers disease.

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Using DNA pooling to screen large samples for candidate gene associations with general cognitive ability. *J.K.J. Kennedy, C.J.C. Curtis, L.M. Butcher, E. Meaburn, R. Plomin.* SGDP, Institute of Psychiatry, London, United Kingdom.

Candidate gene association studies are frequently reported for complex traits and common disorders but frequently fail to replicate in subsequent research. Such studies are often underpowered to detect and to replicate the small effect sizes expected for quantitative trait loci (QTLs) for complex traits and common disorders. DNA pooling of cases versus controls or low versus high groups greatly expedites screening large samples for QTL associations. We used DNA pooling to screen nine genes that have been recently reported to be associated with general cognitive ability (g, often referred to as intelligence), which includes mild mental impairment at its low extreme. From a sample of more than 6000 7-year-old children assessed on four cognitive measures, we conducted two DNA pooling studies in order to replicate our results: (1) a case versus control study in which cases were selected for very low g scores and controls were unselected (n=515 and n=1028, respectively), and (2) a low versus high study (n=503 and 505, respectively). For each of these four groups, five independent subpools were created in order to assess sampling variation. None of the nine candidate genes yielded significant differences in the expected direction in either study. In order to confirm these pooled DNA results, we individually genotyped our entire sample of 6000 children for the most frequently reported association with g, a functional SNP (Val108/158Met) in catechol-O-methyltransferase (COMT). We again found no evidence for association: g scores for the three COMT genotypes were nearly identical and the correlation between g scores and additive genotypic values was .000. We conclude that, although candidate gene association studies are valuable, larger samples and replication designs are needed. In addition, systematic whole-genome approaches using SNP microarrays will provide an important complement to candidate gene studies.

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Molecular Detection of Bacterial DNA in Human Peritoneal Fluid Samples by Broad-Range PCR Amplification.

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Identification of infectious diseases can be difficult when the organisms are problematic to culture. An alternative to culture identification is the use of molecular techniques such as DNA amplification to rapidly amplify specific regions of bacterial genomes followed by direct sequencing of the amplicon. Bacterial DNA can be amplified from human samples using primers specific to bacterial DNA consensus sequences. This study presents preliminary work on development of a broad-range PCR amplification of the 16S rRNA gene sequences for bacteria detection in peritoneal fluid samples. PCR results were compared with results from known standard laboratory culture techniques. DNA was extracted from the 62 peritoneal fluid samples using an alkaline-heat-wash method.

DNA amplification was carried out using primers that targeted the conserved bacterial 16S rRNA gene sequences and which also allowed Gram-typing. Amplified DNA was then directly sequenced and the results submitted for BLAST analysis.

Seventeen peritoneal fluid samples were culture-positive while the remaining 45 showed negative culture results. Concordance between culture-positive and PCR techniques was obtained in only 10 samples. Using PCR and direct sequencing, all 10 bacteria were identified to the species level. Seven culture positive samples were found to be PCR negative. One culture-negative sample obtained from a patient treated with antibiotics was found to be PCR-positive. Overall, the sensitivity of the broad-range PCR assay for amplification of the 16S rRNA gene sequences was 58.82 % in this study.

Further work needs to be carried out to evaluate and optimize the broad range PCR assay for improve sensitivity with peritoneal fluid samples. In addition, more effective rapid DNA extraction and purification techniques should be developed as the insensitive DNA amplification in this study may be due to inefficient DNA extraction and low DNA purity.

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Comparison of Whole-Genome Amplification Methods Using a 10K Microarray Platform. *C.J.C. Curtis, E.L. Meaburn, I.W Craig, R. Plomin.* SGDP research centre, Institute of Psychiatry, London, United Kingdom.

Although immortalization of cell lines yields unlimited amounts of DNA, many large-sample population studies need to rely on DNA extracted from buccal smears for which DNA quantities are much more limited, and re-sampling of DNA stocks is costly and time consuming. Whole-genome amplification (WGA) can extend DNA stocks and provide high yields of unbiased amplification products of the entire DNA template from very small amounts of DNA. In this study we compare four available WGA methods for quantity, quality and accuracy of DNA product produced: (1) primer-extension-preamplification PCR (PEP) which uses random femtomers in a PCR-based reaction; (2) GenomiPhi() and (3) REPLI-g() multiple-displacement amplification, which uses a proofreading enzyme, ()29 DNA polymeraseand (4) GenomePlex(), which converts randomly fragmented genomic DNA into PCR-amplifiable library molecules flanked by universal priming sites. DNA samples for five individuals were amplified using the four WGA methods and genotyped for 10,000 SNPs using Affymetrix GeneChip() Human Mapping 10K Array. Microarray results using amplified DNA were compared to microarray results for the five individuals using original non-amplified DNA. Although the four WGA methods differ in relation to performance, cost and ease of use, the main message is that WGA is a viable technique to amplify DNA.

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High-resolution amplicon melting analysis, a time- and cost-effective method for rapid mutation scanning and frequency studies in the VLCAD gene. *R.K.J. Olsen[1], S. Dobrowolski[2], M. Kjeldsen[1], D.M. Hougaard[3], H. Simonsen[3], C.K. Boesgaard[3], N. Gregersen[1], B.S. Andresen[1, 4].* 1) The Research Unit for Molecular Medicine, University of Aarhus, Denmark; 2) Idaho Technology, Salt Lake City, Utah, US; 3) Department of Clinical Biochemistry, Statens Seum Institut, Copenhagen, Denmark; 4) Department of Human Genetics, University of Aarhus, Denmark.

Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive inborn error of fatty acid oxidation. More than 150 mutations have been identified in the VLCAD gene. Three mutations (779C>T, 830_832del and 848T>C) are frequent in clinically affected patients with VLCAD deficiency and may account for up to approximately 40% of mutant VLCAD alleles in newborns identified by tandem MS based newborn screening. Because newborn screening by acylcarnitine profiling for fatty acid oxidation defects, including VLCAD deficiency, is becoming standard of care in many countries world-wide, there is a need for more time- and cost-effective gene analysis methods for confirmation of screening positive samples. Moreover, knowledge of the carrier frequencies of the most common VLCAD mutations in the general population would allow evaluation of the diagnostic specificity of newborn screening for the disease.

High-resolution melting of PCR amplicons amplified in the presence of the DNA dye LCGreen^{TM} was recently introduced as a homogeneous, closed-tube method for genotyping using the single-sample HR-1^{TM} instrument from Idaho Technology. With the newly developed LightScanner^{TM} from Idaho Technology this method can now been adapted to a 96 well plate format system. Here we validate the LightScanner^{TM} as a mutation scanning instrument by blinded genotyping of >50 sequence variants that we have identified in patients with VLCAD deficiency. We also demonstrate that this method is a very time- and cost-effective method for population carrier testing allowing simultaneous genotyping of three mutations (779C>T, 830_832del and 848T>C) in 2000 Danish blood spots using only one set of primers and the DNA dye LCGreen^{TM}.

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Sequencing the entire mtDNA coding region in LHON patients without Primary LHON Mutations: Biochemical and Molecular Characterization. *K.K Abu-Amero[1], T.M Bosley[2].* 1) Dept Medical Genetics, King Faisal Specialist Hosp, Riyadh, Central, Saudi Arabia; 2) King Khaled Eye Specilaist Hospital.

We investigated the biochemical and molecular characteristics of mitochondria in patients with the Leber hereditary optic neuropathy (LHON) phenotype with and without primary LHON mutations. Patients were selected from neuro-ophthalmology clinic that reported subacute loss of vision in both eyes with optic neuropathies compatible with the LHON phenotype. We reviewed history, clinical examination, and neuroimaging; sequenced the entire mitochondrial DNA (mtDNA) coding region; assessed mtDNA content; and measured mitochondrial respiratory function in some patients and family members. Thirty-five patients (average age at onset 19.0 years; 21 male and 14 female) met diagnostic criteria with median visual acuity 20/200. Six patients (17%) had primary LHON mutations. These patients were all male, and they had increased mtDNA content. The remaining 29 patients did not have primary LHON mutations or increase in their mtDNA content despite a similar clinical presentation, but they had potentially pathologic non-synonymous mtDNA changes (resulting in amino acid changes) significantly more frequently than controls. Seventeen of 19 studied patients had mitochondrial respiratory dysfunction, including two patients with no mtDNA abnormality. In conclusion, the prevalence of primary LHON mutations may be less than generally recognized when patients are selected from a clinical setting rather than from multigenerational families. Nevertheless, patients with the LHON phenotype without primary LHON mutations have evidence of abnormalities involving mitochondrial DNA and respiration, implying that mitochondrial dysfunction plays a role whether or not the primary LHON mutations are present. Complete diagnostic evaluation of these patients could include sequencing the entire mitochondrial coding region and measuring mitochondrial respiratory function.

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A Polymorphism Resource for Genetic Mapping in Zebrafish. *K. Bradley, B. Elmore, J. Breyer, B. Yaspan, J.R. Smith.* Vanderbilt University Medical Center, Departments of Medicine and Cancer Biology, 529 Light Hall, Nashville, TN.

Zebrafish (*Danio rerio*) is a powerful model organism for genetic investigation, enabling rapid mapping of traits in mutagenesis screens. We sought to improve genetic polymorphism maps in Zebrafish by large-scale investigation of sequence data in Sanger databases. Nearly all sequence is sampled from a German laboratory isolate that is not an inbred strain (Tuebingen). Those polymorphisms located within unique genomic sequence are potentially useful for genetic mapping. Roughly one third of the zebrafish assembly harbors non-unique sequence and was used to create a repeat database to enable masking of non-unique clone and shotgun sequence. We employed high-throughput BLAST to compare finished clone sequence to other clones and to shotgun reads to identify all mismatch positions in unique sequence as potential SNPs. We also identified polymorphic STRs on all finished clones. An algorithm assessing the number of instances of each allele allowed us to assign a confidence score for each candidate polymorphism. Empiric sampling of a separate Tuebingen laboratory population and inbred strains (SJA, SJB, and SJC) suggests that over 400K of 600K high confidence SNPs are polymorphic in zebrafish populations publicly distributed by ZIRC for use in the genetic mapping community.

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Fast and reliable mutation screening using Conformation-Sensitive Capillary Electrophoresis. *E. Sistermans, K. van der Donk, E. Bosgoed, M. Ligtenberg, L. Hoefsloot, F. Hol, H. Scheffer.* Dept of Human Genetics, Radboud University Nijmegen Medical Centre, The Netherlands.

Day to day practice in Molecular Genetics Laboratories has seen many drastic changes over the last decade, one of them being the almost complete shift from linkage analysis to mutation analysis. This rapid evolution was made possible by radical improvements of the methods to find mutations either directly by sequencing or by a scanning method. Although large-scale mutation analysis by sequencing is now possible and very accurate, it still is relatively laborious and expensive. Current indirect scanning methods such as DGGE and dHPLC are reliable, but the throughput is limited. We have studied whether Conformation-Sensitive Capillary Electrophoresis (CSCE) using the new POP^{TM} Conformational Analysis Polymer (CAP, Applied Biosystems) might be a fast and reliable alternative for these methods. Using this polymer under semi-denaturing conditions on an automated sequencer (Applied Biosystems 3730) we validated 165 different mutations in 14 genes, including 74 mutations in *BRCA1*, 43 mutations in *CHD7* (CHARGE syndrome) and 9 mutations in the mitochondrial DNA. All mutations were correctly identified due to a shift in the peak pattern. For mitochondrial DNA, mutations present at 10% heteroplasmy could easily be identified. Several parameters were tested to further optimise the method, such as the withdrawal of Urea from the CAP polymer, the running temperature, the addition of GC-clamps, the influence of the length of the fragment and the use of common primers for labelling of the fragments. As CSCE allows the possibility of multiplexing, at least 4 fragments can be simultaneously analysed in each capillary in one sequencer run (2 hours, 50 cm capillary). For a 48 capillary sequencer this results in a throughput of approximately 2300 fragments within a twenty-four hours period. Since the data-analysis is also relatively straightforward, CSCE proves to be a fast and reliable method for routine mutation detection. Furthermore, as no optimization is needed, this technique can also be used for the rapid analysis of candidate genes.

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Analysis of 1,924 disease genes for evaluating utility of functional sequence annotation for mutation screening. *T. Braun[1,2,5], B. O'Leary[5], S. Davis[5], M. Smith[5], H. Almabrazi[5], B. Brown[5], J. Ritchison[5], S. Shankar[2], T. Scheetz[1,2,4], V. Sheffield[3], E. Stone[2], T. Casavant[1,4,5].* 1) Dept Biomedical Engineering, Univ of Iowa, Iowa City, IA; 2) Dept Ophthalmology and Visual Sciences, Univ of Iowa, Iowa City, IA; 3) Dept of Pediatrics, Univ of Iowa, Iowa City, IA; 4) Center for Bioinformatics and Computational Biology, Univ of Iowa, Iowa City, IA; 5) Coordinated Laboratory for Computational Genomics, Univ of Iowa, Iowa City, IA.

The availability of the complete sequence of the human genome has dramatically impacted mutation screening and the search for phenotypically associated sequence variations. In fact, the actual screening of human populations and the subsequent interpretation of observed variations present a larger barrier than the discovery and characterization of candidate genes. We test the hypothesis that some segments of candidate genes are more likely than others to contain disease-causing variations and that these segments can be predicted bioinformatically. A bioinformatic technique (PAR - Prioritization of Annotated Regions) was developed to guide mutation screening based upon functional sequence annotation. In addition, the PAR technique has been optimized using a genetic algorithm and easily scales to additional sequence annotation. This technique was evaluated by using it to analyze 1,924 genes that collectively harbor 27,750 previously published mutations. Nearly 50% of the genes were recognized as disease-associated after screening less than 10% of the complete coding sequence of these genes. The PAR technique identified 90% of the genes as containing at least one mutation with less than 40% of the screening resources that traditional approaches would require. These results suggest that prioritization strategies such as PAR may accelerate disease gene identification through more efficient use of screening resources.

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A newly developed multiplex SNP typing method, DigiTag assay. *N. Nishida[1,2], T. Tanabe[3], M. Takasu[1], Y. Kaneko[2], K. Tokunaga[1].* 1) Department of Human Genetics, The University of Tokyo, Tokyo, Japan; 2) Biomedical Business Incubation Division, Olympus Corporation, Tokyo, Japan; 3) R&D Division, NovusGene Inc., Tokyo, Japan.

Numerous single nucleotide polymorphisms (SNPs) are considered to be candidate susceptibility or resistance genetic factors for multifactorial diseases. Genome-wide search for disease susceptibility regions followed by high-resolution mapping of primary genes require high-throughput, cost-effective and highly reliable technology. At present, there are a variety of SNP genotyping applications, however, many applications need to select relevant SNPs for their assay by in silico assay design, and then a part of candidate SNPs will be excluded from investigation. To accomplish successful typing for all candidate SNPs at a low cost, new technologies must be developed. We developed a new multiplex SNP typing method, named DigiTag assay, and performed typing for 28 SNPs using 40 genomic DNA samples. One of 28 SNPs was revealed to be monomorphic in 40 samples and was therefore excluded from further analysis. This assay was found to be successful in SNP genotyping, giving a high success rate (24 of 27 SNPs) for randomly chosen SNPs. Three SNPs out of 27 SNPs showed indistinct clusters, presumably resulting from miss-ligation in the encoding step (SNP#6 and SNP#9) and insufficient amplification in the multiplex PCR (SNP#19). We demonstrated that mismatch induced 5'f-query probes, which had an artificial mismatch at the fourth position from the SNP base, can sufficiently suppress the false positive signals observed from SNP#6 and SNP#9 without decreasing the positive signals. This newly developed DigiTag assay has the potential to analyze almost all kind of the SNPs with high accuracy and reproducibility by applying mismatch induced 5'-query probes and re-designed primer pairs.

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High frequency of WDR36 missense mutations in a group 301 glaucoma patients from Germany. *F. Pasutto[1], C.Y. Mardin[2], H. Sticht[3], K. Michels-Rautenstrauss[1], B. Rautenstrauss[1], F. Kruse[2], A. Reis[1].* 1) Inst. of Human Genetics, University of Erlangen-Nuremberg, Germany; 2) Dept. of Ophthalmology, University of Erlangen-Nuremberg; 3) Dept. of Bioinformatics, University of Erlangen-Nuremberg.

Mutations in WDR36, a novel gene involved in T-cell activation, on chromosome 5q22.1 (GLC1G) have recently been identified in adult-onset primary-open angle glaucoma (POAG) patients (Monemi, et al. 2005). In our ongoing study on the genetics of glaucoma we now investigated a cohort of 301 unrelated glaucoma patients of German origin for WDR36 mutations. 191 patients presented with POAG, 68 had normal tension glaucoma (NTG) and 42 juvenile-onset primary open-angle glaucoma (JOAG). We screened the entire coding sequence through direct sequencing in all 301 patients and identified a total of 37 different DNA variations. We verified all variants in a group of 96 healthy individuals of comparable age, who had repeated normal ophthalmologic examinations. We classified 10 variants, of which 6 are novel (P31T, D33E, Y97C, H212P, T403A, H411L), as disease causing based on evolutionary conservation of the respective residues in all orthologues and absence from healthy controls. Moreover protein structural analysis based on homology modelling showed that at least some of these WDR36 variations critically affect the stability of the three dimensional structure. These variations were found in 31 patients (10% of total) (23 POAG, 5 NTG, 3 JOAG). Two conserved variants that were previously reported as disease-causing (A449T, D658G) were also found in controls at a comparable frequency (around 1%) and thus most probably represent polymorphisms. Two further variants, previously reported as conferring disease susceptibility (L25P, A163V), were each found in 5 patients but not in controls, further substantiating that they confer risk. In summary, our findings provide further evidence that WDR36 plays an important role in the aetiology of glaucoma. WDR36 gene mutations are associated with adult onset of glaucoma both with high and low intraocular pressure and also with juvenile open angle glaucoma. Supported by DFG Collaborative Research Centre SFB539.

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Molecular changes induced by chronic exposure to the antidepressant paroxetine in a neuronal cell culture system. *M.A. Kennedy[1], P.C. McHugh[1], D.M. Glubb[1], G.R. Rogers[1], P.R. Joyce[2].* 1) Dept Pathology, Christchurch School of Medicine & Health Sciences, University of Otago, Christchurch, New Zealand; 2) Dept of Psychological Medicine, Christchurch School of Medicine & Health Sciences, University of Otago, Christchurch, New Zealand.

Genetic variation may affect individual responses to antidepressant drug treatment. As a means of identifying genes that may be important in antidepressant response, we are exploring the molecular effects of antidepressants in the brain. For this purpose we established a mouse embryonic stem (ES) cell-derived neuronal culture model, and exposed it to the selective serotonin reuptake inhibitor paroxetine. Microarray and proteomic methods were applied to identify genes and proteins undergoing differential expression as a result of chronic (12 day) exposure to paroxetine. Several proteins and genes with synaptic or brain-specific functions were identified in this screen, and the observed expression changes are currently being validated by real-time quantitative PCR and immunoblotting analysis. Expression changes identified in this screening approach may be relevant to antidepressant function and individual drug responses or they may be unrelated bystander effects, and it is necessary to further evaluate the specificity and relevance of the observed expression changes. However, it is possible that a subset of the novel candidate genes and proteins identified here may contribute to the therapeutic effects of paroxetine or provide pharmacogenetic insights into treatment of depression and related illnesses.

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Automation of microarray sample processing using magnetic bead-based SPRI technology. *B. Packard, H. Ebling, E. Gustafson, K. McKernan.* Research & Development, Agencourt Bioscience, Beverly, MA.

Microarray technology is a highly effective and versatile tool for the studying of gene expression, with a wide variety of applications in the pharmaceutical, academic, and clinical research fields. The current trend of microarray technology use is toward multi-sample processing using high, medium, and low-feature density microarray chips arranged in 96 well formats. Sample preparation and processing involves several steps: RNA isolation from various sources, cDNA synthesis, *in vitro* transcription, and generation of labeled probes. Reliable and efficient automation of these steps is required to take full advantage of high throughput array systems. Several column plate-based sample preparation systems are currently available, but are not easily integrated into automated platforms for multiple parallel sample processing. Agencourt Bioscience has developed a group of highly automation friendly magnetic bead reagents using patented SPRI (Solid Phase Reversible Immobilization) technology for the efficient isolation of RNA from cells and tissues, and, a single reagent for the clean up of both cDNA synthesis and in vitro transcription reactions. Agencourts SPRI process allows multiple samples to be easily processed in parallel and offers overall efficiency gains that result in lower total RNA input requirements as well as lower total sample processing costs. In addition, Agencourts magnetic bead sample preparation and processing reagents can be readily integrated into multiple automation platforms. We will present data illustrating an optimized, automated, microarray sample preparation workflow, which demonstrates the capacity of our SPRI magnetic reagents systems to produce high quality, highly reproducible microarray data.

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Identification of NR1I2 genetic variation using resequencing. *C. King[1], M. Minton[1], J. Yu[1], M. Xiao[2], N. Addleman[2], D. Van Booven[1], P. Kwok[2], S. Marsh[1], H. McLeod[1].* 1) Department of Medicine, Washington University School of Medicine, Saint Louis, MO 63110; 2) Cardiovascular Research Institute, Department of Dermatology, University of California, San Francisco, CA, 94143.

Direct activation of NR1I2 occurs in response to a range of xenobiotics, which causes the formation of a heterodimer with RXR. This heterodimer binds to the nuclear receptor response elements and induces expression of genes involved in drug metabolism and transport, including CYP3A4 and ABCB1. This study determined the extent of NR1I2 variation through pooled resequencing in 3 world populations (African, Asian, and European). Of 36 polymorphisms identified, 24 were in the untranslated region, 8 were intronic, and 4 exonic. 36% were unique to the African population and 6% unique to the Asian population. None were specific to the European population. In comparison with previously published data and public database resources, we identified 17 novel polymorphisms. SNPs (NR1I2 -2098, -566, -205, IVS2+55, IVS2+78, IVS6-17, and +1792) were validated in an independent group of 95 African and 95 European subjects. Genotyping was performed using PCR and Pyrosequencing. There was a clear difference in variant allele frequencies between populations. Variant allele frequencies ranged from 0.16 to 0.40 in the European population, and 0.32 to 0.98 in the African population. Of 30 haplotypes, the most common was found at 39% in Europeans, while at 0.68% in Africans. The most common haplotype for Africans (23%) was absent in Europeans. In 52 colorectal caner patients, the NR1I2 genotypes were identical between colon tumor and adjacent normal tissue. Using the Kruskall-Wallis test the 5FR SNP, -566, was associated with CYP3A4 and ABCB1 RNA expression in colorectal tumor samples (p=0.04 in both cases). Polymorphisms in NR1I2 may, in part, explain the considerable inter-individual heterogeneity in ABCB1 and CYP3A4 expression. However, consideration of population differences in allele frequencies should be taken into account when incorporating NR1I2 into pharmacogenetic studies.

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Organization of satellite I and satellite III sequences on human chromosome 21. *R. Hettinger[1], J. Bavarian[1], P. Patel[1], T. George[1], M. Puckelwartz[1], S. McCutcheon[2], M. Cummings[2], J. Doering[1].* 1) Loyola Univ Chicago; 2) Univ Illinois, Chicago.

The human genome sequence does not include the heterochromatic regions, although these sequences comprise 10-15% of the genome. We are constructing a detailed physical map of the HC21 centromere and short arm as a model for the organization of these regions. Our work previously identified two subfamilies of satellite I (sat I) on HC21 which share 80% sequence identity. The N6 subfamily is located solely on the p arm distal to the rDNA cluster, while pTRI-6 sequences are present both in the p arm proximal to the rDNA and in the centromere. The HC21 centromeric sat I cluster has been physically mapped, and portions of it have been sequenced. It is 0.3 Mb long and located within 0.1Mb of the p arm end of the major alphoid cluster, D21Z1. Internally, the sat I cluster consists of tandemly repeated pTRI-6 sequences frequently interrupted by other sat I sequences that have only 80% identity to pTRI-6. These newly-identified sat I sequences are quite distinct from the N6 subfamily or any other sat I sequences in the database, indicating they represent a new subfamily. This centromeric sat I cluster is not found on other chromosomes, and its size and internal organization are polymorphic in the population. It is thus a strong candidate for an HC21-specific centromeric marker. Satellite III (sat III) sequences are found on the p arm of HC21 both proximal and distal to the rDNA. We have isolated three new sat III sequences (2.5-4.4 kb in length) from a HC21 specific library, and through the use of an HC21 hybrid cell mapping panel, find them to be located proximal to the rDNA. Sequencing reveals that all three are members of sat III subfamily I, which is characterized by high percentages of the typical GGAAT monomer repeat and very low levels of the GGAGT variant repeat. The sequences have highly heterogeneous organizations with no obvious higher order repeats. BLAST comparisons showed that the three new sat III sequences have less than 80% identity to each other or with any other currently known sat III sequence in the human genome. Thus, they are candidates for HC21p-specific probes.

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Characterization of the chAB4 duplicon on proximal 21p. *E. Miller[1], N. Alvi[1], K. Arendes[1], L. Chen[1], R. Ganith[1], M. Cummings[2], J. Doering[1].* 1) Loyola Univ Chicago; 2) Univ Illinois, Chicago.

While 10-15% of the human genome is composed of heterochromatic DNAs, these regions are not included in the completed sequence. We are creating a detailed physical map of the centromere and p arm of HC21 as a model for the organization of such regions. Our previous work identified a number of low copy number (LCNR) sequences in the centromere of HCY and on the proximal short arm of HC21p. These sequences have a very similar linear order on both HC21 and HCY, except that the order on HC21 is inverted relative to that on HCY. We have now sequenced 9 of these LCNRs ranging in size from 663bp to 4,856bp. BLAST analysis indicates that at least 5 of these LCNRs are part of the larger chAB4 duplicon. The chAB4 duplicon is ~86kb in length and is composed of a chAB4 DNA segment adjacent to a 48bp satellite array of variable length, followed by an NF1-related pseudogene. While others work suggested that chAB4 has a palindromic organization, sequence analysis of a number of loci containing the chAB4 duplicon and adjacent LCNRs shows no evidence that it is part of a palindrome on HC21 or HCY. This data clarifies the organization in a previously uncharacterized region of the centromere on HCY. On HC21 a full copy of the chAB4 duplicon and adjacent LCNRs is found on the proximal p arm in the 21-II region. The 21-II region is composed of monomeric alphoid, LINEs, and other satellite DNAs. Using BLAST, we identified BACs containing chAB4 duplicon sequences that had not been placed in the genome map. By comparing these BAC sequences, copies of chAB4 on HC21, Y, 17, and 22 showed at least 90% similarity. This work indicates that chAB4 may actually be part of a larger duplicon ~200kb in size. Heterogeneity in the duplicon structure is indicated by a ~25kb insertion into the sequence on HCY and HC17. BACs containing chAB4 and adjacent LCNRs located in the proximal p arm of HC21, as well as BACs found at the distal end of the D21Z1 region containing monomeric alphoid DNA and satellite III, will allow closing the gap between the two regions. This will create a contiguous map of ~5Mb that links the 21-II and D21Z1 regions, joining the p arm to the q arm.

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Detecting Polymorphisms in Drug Metabolism Genes Uncovers More than Genotypes. *W. Tom, S. Liew, T. Harkins, D. Ingber, C. Chiang, Z. Gu, S. Cai, R. Charlab, Y. Wang, K. Lazaruk.* ART, Applied Biosystems, Inc, Foster City, CA.

Polymorphisms within the drug metabolism pathways have been difficult to study due to the complexity of genetic information associated with these genes. Many of the genes within these pathways are part of large gene families that include several pseudogenes, thus generating large homology barriers that are difficult for most technologies to overcome. Additionally, many of the polymorphisms are identified in public SNP databases that do not use common genomic identifiers that allow researchers to readily understand which polymorphisms they are dealing with. To overcome these barriers, Applied Biosystems has developed an extensive collection of TaqMan Genotyping Assays to detect SNPs, multiple nucleotide polymorphisms (MNPs), and insertion/deletions (in/dels) within 220 drug metabolism genes. The polymorphisms identified for this collection are within regulatory elements, coding regions, or splice junctions. Each assay was wet-lab tested on 180 DNA samples from 4 human populations, (Caucasian, African American, Chinese and Japanese) to estimate the minor allele frequency in each of these populations and all assays were checked for Hardy-Weinberg Equilibrium. During the performance testing of the assays, many of the genes in this collection were identified as having unique characteristics, for example, gene deletions or amplifications (GSTM1 and CYP2D6) that can affect the interpretation of results from SNP genotyping assays. It is important to understand the underlying biology of particular genes when interpreting results from genotyping experiments, and when evaluating how well a particular genotyping technology performs.

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Sequence context analysis of single nucleotide polymorphisms (SNPs) in human CpG islands. *Z. Zhao*[1, 2]. 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 2) Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA.

A detailed examination of the non-random sequence context where the genetic polymorphism occurs is important for understanding the mechanism of mutation, protein-DNA interaction and genome sequence evolution. CpG islands are commonly found in the promoter regions of the genes in vertebrate genomes and involve in the regulation of gene expression and cell differentiation by methylation mechanism. In this study, we first searched for CpG islands in the human genome using the CpG island searcher program (CpGi130) and then categorized SNPs (dbSNP build 123) in the CpG islands by matching the position of a SNP with the location of a CpG island in a contig sequence. We identified 34,928 putative CpG islands in the human genome and located 133,591 SNPs within these islands. The proportion of nucleotides, CpG dinucleotides and CpG SNPs in the CpG islands on each chromosome correlated with the GC content of the chromosome. In the CpG islands, the frequency of CpG dinucleotides at the polymorphic sites was nearly the same as that in the CpG island sequences, although this was 6.09 times that in the human genome sequences. Moreover, CpG SNPs were 3.92-fold less prevalent relative to the presence of CpG dinucleotides in the CpG islands. Finally, an analysis of SNPs in the mouse CpG islands revealed the similar patterns, though the degree was somewhat different between the two species. These results provide strong evidence for the suppression of the nucleotide changes at CpG dinucleotides in the CpG islands and the hypermutability of methylated CpG dinucleotides in the non-CpG island sequences in the human and mouse genomes.

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Identification of SNPs with metabolic impact. *N.J. Marini[1], J.S. Ziegle[2], K.L. Hunkapiller[2], D.G. Ginzinger[2], P.D. Thomas[2], D.A. Gilbert[2], J. Rine[1].* 1) Molecular and Cellular Biology, Univ. of California, Berkeley, CA; 2) Applied Biosystems, Inc., Foster City, CA.

Compelling data from human and bacterial genetics show that the phenotype of some mutations in vitamin-dependent enzymes can be suppressed by vitamin supplementation. Such vitamin-remedial mutations can affect the binding site for the vitamin cofactor, or can destabilize the protein in a way that higher vitamin concentration can remedy. The few known cases from human genetics are rare and typically result in severe disease that is vitamin remedial. To test the hypothesis that there may be polymorphisms that more subtly affect enzyme activity, yet are vitamin-augmentable by the same principles, we have begun a re-sequencing study on 4 prototypical genes in a large (564 individual), ethnically diverse population. All missense variations from Ornithine Aminotransferase (OAT), Thymidylate Synthase (TYMS), Methylenetetrahydrofolate Reductase (MTHFR) and Glycinamide Ribonucleotide Transformylase (GART) are being evaluated in functional assays based on complementation in the yeast, Saccharomyces cerevisiae. Thus far, we have completed sequence analysis for 14 of 33 total coding exons (comprising parts of each of the four genes). We have identified 12 novel low frequency coding variants (allelic frequencies <.003) with at least one variant seen in each of the four genes. The sum total of such alleles potentially indicates a significant contribution to functional variation in the population. The current status of variants identified and functional assessment will be presented as well as the implications for the broader genomic set of vitamin-dependent enzymes.

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TaqMan based SNP genotyping using whole genome amplified products from FTA blood samples. *G. Sun, R. Kaushal, P. Pal, H. Cheng, Y. Shen, J. Mallik, R. Deka.* Center for Genome Information, Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

FTA cards provide a convenient medium for collection, storage and shipment of blood samples for DNA analysis. However, the amount of extractable genomic DNA is limited for use in large-scale typing of genetic markers. Whole genome amplification (WGA) has emerged as a fundamental method for reproducing abundant quantities of DNA. Two of the widely used WGA methods include improved-primer extension preamplification (I-PEP) and multiple displacement amplification (MDA). We have evaluated the relative efficiencies of these two methods in amplifying DNA obtained from blood spots collected on FTA cards for high-throughput single nucleotide polymorphism (SNP) analysis. We used the TaqMan assay to type 27 SNPs using genomic DNA extracted from 30 FTA cards and their corresponding I-PEP and MDA products. Using the I-PEP protocol, we obtained high fidelity of amplification and allelic discrimination in 23 of the 27 markers (85%), whereas only 8 of the 27 markers (30%) could be comparably scored using MDA products. Compared to FTA genomic DNA, MDA products showed 7 mismatches out of 240 genotypes (2.9%) while I-PEP showed only 1 mismatch in a total of 690 genotypes (0.14%). We computed the sensitivity and specificity rates for both protocols by fixing the Quality-value at 90% in the SDS software of the 7900HT machine. The sensitivity rate for I-PEP was 82.7% compared with 28.8% for MDA. The specificity rates for I-PEP and MDA were 96.4% and 79.4%, respectively. Our results demonstrate relative advantage of the I-PEP protocol in the analysis of SNP markers from FTA blood spots. WGA products obtained from a single punch of the FTA card (1.2 mm, approximately 0.5~1 ml of blood) could be sufficient for ~750 PCR reactions.

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Automated Preparation and Analysis of Multiplex Mutation and SNP panels. *N. Udar, J. Mariana, J. Parker, D. Clark, L. Pajak, T. Reyes, S. Boyer.* Beckman Coulter Inc., Fullerton, CA.

Genotyping using Single Nucleotide Polymorphisms (SNPs) has become common in areas such as linkage/association studies, pharmacogenetics, forensics, etc. Single Base Extension (SBE), the gold standard technology for SNP genotyping, provides a robust solution in a multiplexed format. Although high throughput SNP detection platforms are available, there is significant demand for medium throughput capabilities, especially on a time tested mainstream capillary electrophoresis platform. We have used SBE technology to extend an unlabeled primer with a labeled terminator to detect base changes in the DNA. Single nucleotide changes, deletions and insertions can be scored by this technique using a single primer extension reagent mixture. An important consideration is that there are no additional secondary steps like ligation or enzymatic cleavage after the extension step. The products are treated with SAP and loaded on a capillary electrophoresis system for detection. We demonstrate multiplex capability using SNP panels of at least 10 SNPs. A multiplex cystic fibrosis (CF) mutation panel was also tested using patient samples. The CF panel of mutations among others includes deltaF508, G85E, N1303K, R117H, 3659delC. Signal balance between the two heterozygous alleles is crucial for accurate detection. We measured this signal balance for each of the two alleles of the 6 possible heterozygote combinations and found an average signal ratio of 1:1.6. The allele calling accuracy for a 10-plex reaction is 99%. An automated DNA extraction step on a robotic platform was then integrated to increase process performance, reduce possible sampling errors and hands on time per sample. Tests were also carried out to determine capillary array life and our results demonstrate that the capillary life can significantly be extended due to the reduced time in the run method. Comparisons were done using different sources of DNA including DNA extracted from cell lines and those prepared from whole genome amplification. Our results demonstrate an efficient process of producing more than 960 SNP/mutation screens per plate on a 8-capillary electrophoresis platform.

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Identification of transcription factor bindings sites at base pair resolution by chromatin immunoprecipitation and genomic microarrays. *C. Wadelius[1], A. Rada-Iglesias[1], O. Wallerman[1], C. Koch[2], A. Ameur[3], S. Enroth[3], G. Cleland[2], S. Wilcox[2], O. Dovey[2], P. Ellis[2], V. Wraight[2], K. James[2], R. Andrews[2], C. Langford[2], P. Dhami[2], N. Carter[2], D. Vetrie[2], J. Komorowski[3], I. Dunham[2].* 1) Dept Gen/Pathology/Rudbeck Lab, Uppsala Univ, Uppsala, Sweden; 2) Sanger Institute, Cambridge, UK; 3) Linnaeus Centre for Bioinformatics, Uppsala, Sweden.

Introduction: In a genomic perspective very little is known of how gene activity is regulated. One objective for the ENCODE project is to evaluate methods to characterise gene regulatory networks in 1% or 30 Mb of the human genome. Methods: Antisera against HNF3b, HNF4a, USF1 and acetylated histone H3 were used in chromatin immunoprecipitation to enrich DNA interacting with each protein in the liver cell line HepG2. Precipitated DNA and reference DNA were differentially labelled and hybridised to a high resolution tiling path genomic array of ~19500 PCR fragments covering 75% of the ENCODE region and positive spots were identified. Results: HNF3b and HNF4a are frequent regulators of gene activity in HepG2 cells, with 154 and 194 binding sites respectively. USF1 is more specialised with 31 binding sites. Many sites are in 5 ends of genes but presumed regulatory sequences are frequently also found in intronic and intergenic regions. Acetylated histones are mostly found at 5 ends of genes. Bioinformatic analysis of the sequences bound by each TF shows an overrepresentation of motifs highly similar to the in vitro established consensus sequences. Based on these data we have identified tentative binding sites at bp resolution. Some of these sites have previously been found by in vitro analysis and others were verified in vitro in this study. It is likely that positive signals are generated in every ChIP-chip study as a consequence of indirect DNA-protein interaction but by this strategy it is possible to identify some of them. Conclusion: It is possible to identify at least some TF binding sites at bp resolution in a genomic ChIP-chip analysis and to distinguish between direct and indirect DNA-protein interaction. This strategy can be used for many other transcription factors and may be scaled up to still larger parts of the genome.

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Comparative genomic analysis identifies endothelial cell-specific gene conserved noncoding sequences (EC-CNSs) that are DNase hypersensitive. *J.A. Bernat[1], A.S. Kondrashov[2], G.E. Crawford[3], F.S. Collins[3], D. Ginsburg[1,4,5].* 1) Dept. of Human Genetics, Univ. of Michigan, Ann Arbor, MI; 2) NCBI, NIH, Bethesda, MD; 3) NHGRI, NIH, Bethesda, MD; 4) Dept. of Internal Medicine, Univ. of Michigan, Ann Arbor, MI; 5) HHMI, Univ. of Michigan, Ann Arbor, MI.

Von Willebrand factor (VWF), a key blood clotting protein, is a standard histologic marker for endothelial cells. Previous studies examining the proximal promoter region have failed to fully recapitulate the endogenous pan-endothelial VWF expression pattern. To identify potential novel *VWF* distal regulatory elements, as well as a more general endothelial-specific expression program, we aligned human and mouse orthologous genomic sequences from *VWF* and 27 other endothelial-specific genes, extending from the neighboring 5-upstream gene to the end of intron 1 (excluding the core promoter and exon 1). We analyzed the resulting 262 EC-CNSs by quantitative PCR for hypersensitivity to DNase I digestion. 33% of the EC-CNSs (including 6 of 9 upstream of *VWF*) were hypersensitive (HS) in human umbilical vein endothelial cells (HUVECs), while only 16% were HS in control human fibroblast cells. 20% of the EC-CNSs were HS in HUVECs only, suggesting that they may identify novel endothelial-specific transcriptional elements. When the 262 EC-CNSs were compared to CNSs from non-endothelial genes, a single motif, AGGAAR, was identified as overrepresented in EC-CNSs. This association was replicated in an independent set of 28 additional genes with high HUVEC expression. The motif was also overrepresented in the subset of DNase HS EC-CNSs. The AGGAAR motif includes the core binding sequence (GGAW) of the Ets family of transcription factors, which contains several members implicated in tissue-specific gene expression. These results suggest that the AGGAAR motif may represent the target sequence for a specific Ets family member critical for determining the endothelial-specific gene expression program. We are currently testing this hypothesis by analyzing this motif in *Vwf* BAC transgenic mice containing 177kb of upstream sequence, including all nine identified EC-CNSs.

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Quantitative gene expression profiling of multiple toxicity mechanisms in chemically-treated, liver-derived rat cells using a toxicity panel of 25 genes. *J. Luo[1], H. Chi[1], G. Jackson[1], S. Joo[1], K. Oades[2], P. Pezzoli[2], G. Vansant[2], S. Boyer[1].* 1) Beckman Coulter Inc; 2) Althea Technologies Inc.

Gene expression profiling analysis is becoming one of the most frequently used tools for functional genomics. Here, we describe an optimized gene expression profiling system that utilizes a patented amplification strategy with both gene-specific and universal primers designed by a proprietary multiplex primer design algorithm. After multiplex reverse transcription and a few initial PCR cycles, the universal primers, one of which is labeled with a fluorescent dye, amplify multiple target fragments simultaneously and uniformly. Therefore, gene transcript ratios in RNA samples are maintained during the multiplexed RT-PCR amplification process. The multiplex primers are optimized to generate amplified, dye-labeled fragments that are well separated eletrophoretically in a size range of 100-400 nucleotides for unambiguous gene identification. The amplification products are well balanced within a linear detection range of the CE system for precise signal quantification. Reference genes are included to allow peak signal normalization for relative quantitative analysis. With this design interface, a rat toxicity panel, called Rat MultitoxPlex, was developed targeting 25 genes, most of which are involved in different toxicity pathway elements. The quantitative assay compares variation in cellular transcription profiles, as end-points of different toxicity mechanisms or pathways. RNA samples of 20-25 nanograms from compound-treated and un-treated rat hepatocytes were amplified using MultitoxPlex reagents. Amplified, dye-labeled fragments were separated by CE. Gene-specific peaks were identified, quantitated, normalized, and gene expression profiles created and visualized using our proprietary software. The results illustrate the ability of the MultitoxPlex to accurately capture the gene expression changes caused by compound treatment, lending insight to their impact on specific mechanistic toxicities in cells. We find this approach to be a cost-effective, automated alternative for acquiring sensitive and accurate information of quantitative gene expression profiles.

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Automated verification of predicted tissue and tumor specific splice isoforms by Microfluidic separations of RT-PCR products. *M. Greiner[1], R. Salowsky[1], D. Zink[2], S. Haas[2], B. Korn[2].* 1) Agilent Technologies, Waldbronn, Germany; 2) RZPD Ressourcenzentrum für Genomforschung, Heidelberg, Germany.

Alternative splicing of transcripts may lead to different mRNA species and therefore to potentially different proteins. Any failure or error in the splicing control mechanism can be involved in a number of pathological processes, such as cancer. Therefore, splice isoforms that are disease specific could serve as excellent diagnostic markers, which are easily identifiable by PCR. Computational prediction of alternative splice variants has been highly facilitating the identification of novel splice isoforms. Our prediction strategy is based on the genomic mapping (SpliceNest) of EST consensus sequences and library annotation provided in the GeneNest database. This revealed 427 genes with at least one tissue specific transcript as well as 1120 genes showing tumor specific isoforms. Out of these genes, a subset of predicted isoforms were experimentally verified by an RT-PCR screening approach. We have set up an experimental strategy that allows us to screen expression of genes in up to 112 different human tissues of multiple developmental stages and cell lines. To circumvent the bottleneck of DNA separation using slab gel analysis, an automated on-chip electrophoresis system that allows unattended high throughput analysis of DNA fragments was implemented in the workflow. In our experimental set-up, we analyzed RT-PCR samples on 4 x 96 well plates within a defined sequence of consecutive one-on-one measurements. The high throughput experimental verification of computationally predicted tissue specific isoforms revealed a high success rate in confirming their expression in the respective tissue.The combination of computational prediction of alternative splicing events with high throughput microfluidic verification facilitates the efficient detection of tissue and tumor specific transcripts.

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HOXA13 In Vivo Regulatory Binding Sites. *C.D. McCabe[1], J.W. Innis[1,2].* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Pediatrics, University of Michigan, Ann Arbor, MI.

Few authentic, regulatory binding sequences in mammalian genomic DNA are known for the 39 mammalian HOX transcription factors. To further our understanding of gene regulation by HOX proteins we sought to identify HOXA13 DNA binding sites in vivo using chromatin immunoprecipitation (ChIP) in a 3T3-derived cell line that stably expresses nuclearly-localized, FLAG-tagged HOXA13. We have published that numerous endogenous genes are reproducibly up- or down-regulated in these cells. Using anti-FLAG ChIP we isolated and sequenced 85 randomly chosen genomic ChIP clones and prioritized 21 for reproducibility testing in five subsequent independent ChIP experiments. 33% of the tested fragments were enriched in 5 experimental replicates. One of these fragments is located within intron 2 of Enpp2. ENPP2, also known as autotaxin, is known to modulate tumor and normal cell motility and expression is highly upregulated by HOXA13 in these cells. Reporter gene expression constructs were minimally enhanced in cotransfections with HOXA13 by including this fragment, suggesting that alone this HOXA13-ChIP fragment is insufficient to promote the high level of Enpp2 expression induced in these cells. Taking a candidate approach, three out of four additional candidate HOX binding sites within the Enpp2 promoter region were highly enriched in ChIP. A deletion/point mutation set of Enpp2 promoter fragments is being tested for response to HOXA13 co-transfection. Our studies have discovered novel, genomic binding sites for HOXA13 through a non-candidate approach and for one target gene show a direct correlation between induced expression of Enpp2 and association with the promoter and gene chromatin by HOXA13.

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L1 Integration in a Transgenic Mouse Model. *D.V. Babushok[1], E.M. Ostertag[1,2], C.E. Courtney[1], H.H. Kazazian, Jr[1].* 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA.

In the race against mutational inactivation, choosing an integration site that permits transcription, yet is dispensable for the hosts survival, is key to a retrotransposons evolutionary success. To study integration preferences of the human LINE-1 retrotransposon (L1), we developed a transgenic mouse model of L1 retrotransposition that displays both germline and somatic *de novo* L1 insertions at a high frequency. We mapped 3 integration sites of 51 *de novo* L1 insertions by Thermal Asymmetric Interlaced PCR (TAIL-PCR). Analysis of integration locations with respect to genomic landmarks revealed a generally broad distribution with a modest preference for intergenic regions. We characterized the complete structures of 33 of these *de novo* retrotransposition events. Our results highlight the tremendous number of highly truncated L1s, with over 52% (27/51) of total integrants less than 1/3 the length of a full-length element. New integrants carry structural characteristics typical of natural genomic L1 elements, with a number having inversions, deletions, and 5-end microhomologies to the target DNA sequence. Notably, at least 13% (7/51) of all insertions contain a short stretch of extra nucleotides at their 5 end, including various regions of identity to sequences surrounding the insertion target site. Similar 5 extra nucleotides have been described previously for LINE-like elements. We propose that most of these unexplained nucleotide additions in the context of 5-truncated L1s can be accounted for by a combination of 1) template-jumping of the L1-encoded reverse transcriptase onto the cleaved host DNA 5 to the elements integration site, 2) very short inversions, and 3) reverse-transcriptase mediated insertion of a few untemplated nucleotides. These data represent the first characterization of a large number of *de novo* L1 integrants *in vivo*.

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Rho GTPases Expression in embryonic rat cortical neurons induced by phenylalanine. *X. Gu, Y. Zhang.* Shanghai Inst Pediatric Res, Xin Hua Hosp, Shanghai, Shanghai, China.

Phenylketonuria is characterized by elevated blood phenylalanine levels and mental retardation. Rho GTPases are key signaling proteins that regulating neurite growth and synaptic connectivity. To understand the mechanisms of phenylalanine neurotoxicity, the role of Rho GTPases in neuronal injury induced by phenylalanine has been examined. After 2 days and 4 days exposure to phenylalanine (0.9mM), cultured embryonic cerebral cortical neurons were fixed and double-immunostained with a polyclonal antibody against MAP2 and a monoclonal antibody against Tau-1. The results demonstrated that phenylalanine reduced primary dendrite and its branch number and spine density in cultured neurons induced by phenylalanine. Real time PCR and Western blot analysis were used to evaluate the Rho GTPases (Rac1, Cdc42, RhoA) mRNA and protein expression. We discovered that phenylalanine downregulated Rac1, Cdc42, RhoA mRNA and protein expression. Active Rho GTPases were pulled down by GST¨CPBD or GST¨CRBD. Western blot showed that phenylalanine decreased Cdc42/Rac activity with time dependent, but increased RhoA activity. These results may provide important insights into the molecular mechanism underlying abnormality of dendrite and dendrite spine induced by phenylalanine, and probably is one of complicated mechanism of neuronal injury induced by phenylalanine.

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Intraallelic variability in the R408W phenylketonuria mutant lineages in Europe. *D. Croke[1], O. Tighe[1,2], G. Bertorelle[3], C. O'Neill[2], E. Naughten[2], P. Mayne[1,2].* 1) Dept Biochemistry, Royal Col Surgeons, Dublin, Ireland; 2) Department of Pathology, The Children's University Hospital, Dublin, Ireland; 3) Dipartimento di Biologia, Universita di Ferrara, Ferrara, Italy.

The common Phenylketonuria mutation in Europe, R408W, is observed on chromosomes of two distinct haplotypes as the result of recurrent mutation: the two lineages are referred to as R408W-2.3 and R408W-1.8. Spatial autocorrelation analysis of their frequency distributions suggests that R408W-2.3 was dispersed across central and eastern Europe by human migration while R408W-1.8 arose in Ireland with limited subsequent spread [O Donnell et al., Eur. J. Hum. Genet. 2002, 10: 530-538; Tighe et al., Hum. Mutat. 2003, 21: 387-393]. This study aimed to investigate the two R408W lineages further to assess allele ages and the effect that selection may have played in their population history. Three novel dinucleotide Short Tandem Repeat (STR) markers within and flanking the human Phenylalanine Hydroxylase (PAH) locus were identified by in silico methods. These markers were typed by standard PCR-based methods in a set of anonymised patient and control DNA samples (n=182) from fifteen European populations genotyped previously for the markers used traditionally to determine PAH haplotypes [7 SNPs, 1 VNTR and 1 tetranucleotide STR]. This sample set included individuals of wild-type (n=49), R408W homozygote (n=75), compound heterozygote including R408W (n=16) and R408W heterozygote (n=8) genotypes. Haplotypes were reconstructed using the PHASE v2.02 software. Phylogenetic analysis based upon STR haplotypes produced unrooted star-like networks demonstrating comparable levels of diversity for the R408W-2.3 and R408W-1.8 lineages. Both networks were centered on a major haplotype [61% of R408W-1.8 alleles; 49% of R408W-2.3 alleles] and contained a total of 17 mutational steps. Estimates of allele age based upon intra-allelic variation generated values of 4,850 - 37,975 yBP for R408W-1.8 and 7,700 - 44,050 yBP for the R408W-2.3 mutation. Neutrality tests suggested that the two R408W lineages increased in frequency over time due to population expansion rather than heterozygote advantage.

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Moyamoya syndrome in a patient with concomitant mitochondrial and fatty acid oxidation disorders. *N. Longo[1], I. Schrijver[2], H. Vogel[2], L.M. Pique[2], T.M. Cowan[2], M. Pasquali[1], G.L. Hedlund[1], S.E. Ernst[1], R.C. Gallagher[2], G.M. Enns[2].* 1) Univ Utah, Salt Lake City, UT; 2) Stanford Univ, Stanford, CA.

MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) is a maternally inherited disorder characterized by recurrent cerebral infarctions that do not conform to discreet vascular territories. Here we report a patient who presented at 7 years of age with loss of consciousness and severe metabolic acidosis following vomiting and dehydration. She developed progressive sensorineural hearing loss, myopathy, ptosis, short stature, and mild developmental delays after normal early development. Biochemical testing identified metabolites characteristic of Medium Chain Acyl CoA Dehydrogenase (MCAD) deficiency (hexanoylglycine and suberylglycine), but also severe lactic acidemia (10-25 mM) and, in urine, excess of lactic acid, intermediates of the citric cycle, and marked ketonuria, suggesting mitochondrial dysfunction. She progressed rapidly to develop temporary cortical blindness. Brain imaging indicated generalized atrophy, more marked in the left side, in addition to white matter alterations consistent with a mitochondrial disorder. Magnetic resonance angiography indicated occlusion of the left cerebral artery with development of collateral circulation (Moyamoya syndrome). This process aggravated over time to involve the other side of the brain. A muscle biopsy indicated the presence of numerous ragged red fibers. Molecular testing confirmed compound heterozygosity for the common mutation in the MCAD gene (985A>G) and a second pathogenic mutation (233T>C). mtDNA testing indicated that the muscle was almost homoplasmic for the 3243A>T mutation in $tRNA^{Leu}$, with a lower mutant load (about 50% heteroplasmy) in blood. 3243A>T $tRNA^{Leu}$ has been previously associated with MELAS and severe encephalopathy. These results indicate that mitochondrial disorders may be associated with severe vascular disease resulting in Moyamoya syndrome, and angiography may be indicated in MELAS since Moyamoya syndrome may be improved with surgery. The contribution of the concomitant MCAD deficiency to the development of the phenotype in this case is unclear.

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A 32 y/o male with MMA, progressive dystonic spastic diplegia, cystic encephalomalacia of the globus pallidum and Wallerian degeneration. *D. Adams[1], J. Sloan[1], U. Sundaram[4], E. Baker[3], A. Gropman[2,5], C. Venditti[1].* 1) NHGRI, NIH, Bethesda, MD; 2) Center for Functional and Molecular Imaging, Georgetown U., Washington, D.C; 3) CC/DRD, NIH, Bethesda, MD; 4) Virginia Commonwealth U., MCV, Richmond,VA; 5) NINDS, NIH, Bethesda MD.

We present a 32 y/o male with B12-responsive methylmalonic aciduria (MMA) and progressive dystonic spastic diplegia. He was in good health and developing appropriately until 13 months of age when he suffered a catastrophic neurological crisis with encephalopathy leading to coma. He was afterward diagnosed with MMA and began oral cyanocobalamin. Due to presumed static encephalopathy and spastic diplegia, he carried a diagnosis of cerebral palsy for two decades. He was ambulatory, ran, and participated in the Special Olympics. Over the past 10 years, he experienced increasing difficulty with ambulation and worsening spasticity, necessitating use of a walker. Brain MRI performed at 3T on a GE magnet disclosed focal increased T2 signal in the globus pallidum adjacent to the posterior limb of the internal capsule, and cystic encephalomalacia of the remainder of the nucleus. There was also suggestion of Wallerian degeneration. MRS of gray, white, and CSF territories performed at long and short TE did not demonstrate lactate elevation. Initial labs showed a serum B12 level of 5507 pg/ml, a homocysteine level of 8 umol/L, and a urine MMA excretion 694.7 mg/24 hrs. Approximately 24 hours after a single intramuscular dose of hydroxycobalamin (1 mg), the urine B12 excretion dropped to 275.6 mg/24 hrs. In summary, important features in this case are functional motor decline, despite the apparent lack of metabolic crises, and metabolic stability with oral cyanocobalamin therapy alone. The MRI findings provide an illustration of the pathological evolution of a remote metabolic infarction of the globus pallidus culminating in cystic replacement. The Wallerian degeneration of the corticospinal tract suggested by neuroimaging may partially explain the progressive nature of motor symptoms seen in a subset of patients with MMA s/p metabolic stroke. Additional neuroimaging is planned to better understand this patients pathophysiology.

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Intracellular galactose-1-phosphate accumulation leads to environmental stress response in yeast. *K. Lai[1], M. Tang[2], T. Slepak[1].* 1) The Dr. John T. Macdonald Foundation Center for Medical Genetics, Dept Pediatrics, University of Miami SOM, Miami, FL; 2) Dept Biochemistry and Molecular Biology, University of Miami SOM, Miami, FL.

Inherited deficit of galactose-1-phosphate uridyltransferase (GALT) can lead to the potentially lethal disorder Classic Galactosemia. Although the biochemical defects associated with GALT deficiency have been described in detail in patients and various model organisms, few attempts have been made to characterize the pathogenic mechanisms of Classic Galactosemia at the molecular level. Here we report the use of high-throughput DNA microarrays to examine how galactose affects gene expression in isogenic yeast strains that are deficient in either galactokinase (GALK) or galactose-1-phosphate uridyltransferase (GALT), two enzymes which are essential for normal galactose metabolism. We confirmed that the growth of our GALT-deficient, but not GALK-deficient yeast strain ceased 4 hours after challenge with 0.2 percent galactose. Such inhibition was not associated with a reduction of ATP content, and was reversible after removal of galactose from medium. We compared the gene expression profiles of the GALT-deficient and GALK-deficient cells in the presence/absence of galactose. We revealed that in the absence of galactose challenge, a subset of genes involved in RNA metabolism was expressed at a level 3-fold lower in the GALT-deficient cells. Upon galactose challenge, significantly more genes involved in various aspects of RNA metabolism and almost all ribosomal protein (RP) genes were down-regulated in the GALT-deficient, but not GALK-deficient cells. Remarkably, genes involved in inositol biosynthesis and turnover were exclusively induced at high level in the galactose-intoxicated, GALT-deficient cells. Our data thus suggested that RNA metabolism, ribosome biogenesis and inositol metabolism were likely targets for galactose-1-phosphate, a toxic intermediate that is uniquely accumulated under GALT-deficiency.

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Classic Galactosemia: A Paradigm for Endoplasmic Reticulum Stress Response. *T. Slepak, K. Lai.* The Dr. John T. Macdonald Foundation Ctr. for Med. Genetics, Ped. Dept., U of Miami, Miami, FL.

In humans, deficiency of galactose-1-phosphate uridyltransferase (GALT) can lead to the potentially lethal disorder Classic Galactosemia. Although the biochemical abnormalities associated with this disease are well-characterized, few attempts have been made to delineate the pathogenic mechanisms of the disorder at the molecular level. Here we report the use of high-throughput DNA microarrays to examine how galactose challenge affects global gene expression in primary fibroblasts derived from 3 un-related galactosemic patients. To control for genetic variation among individuals, we compared the gene expression profiles of the 3 patient cell lines infected with a lentiviral vector expressing the GALT gene (*tests*) with the corresponding patient cell lines infected with a lentiviral vector expressing the green fluorescent protein (GFP) (*controls*). Consequently, 3 pair-wise comparisons were resulted from the 3 patient lines that had been challenged with 0.1%; galactose for 48 hours. We focused on the genes whose expression level was changed over 2-fold in a similar manner in all three comparisons. In the controls, we detected an 11-fold increase in the expression level of the BiP gene, which encodes the major ER stress sensor protein. Expression of genes that encoded numerous ER-resident molecular chaperones, foldases, proteases and lectins were also up-regulated at a level of 3-fold or higher in these cells. To confirm the effect of galactose on ER function in the galactose-challenged, GALT-deficient fibroblasts, we examined the functional expression of epidermal growth factor receptor (EGFR), a glycoprotein with 11 *N*-linked glycosylation sites, in the controls. Despite a 3-fold up-regulation of EGFR mRNA level, we saw a sharp reduction in the EGFR protein in the controls when compared to the tests. Our findings were highly significant because not only did they show, for the first time, that galactose challenge in human GALT-deficient cells led to ER stress, they also unveil novel molecular targets/signaling pathways that were affected by the *in vivo* accumulation of toxic metabolites under GALT deficiency, notably galactose-1-phosphate and galactitol.

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Atypical presentation of citrin deficiency. *D. Dimmock[1], K. Kobayashi[2], M. Iijima[2], A. Tabata[2], T. Saheki[2], B. Lee[1], F. Scaglia[1].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Molecular Metabolism & Biochem Gen, Kagoshima University, Kagoshima, Japan.

Citrin deficiency is a rare inborn error of metabolism previously seen in Southeast Asian patients. It is caused by mutations in the *SLC25A13* gene that encodes an aspartate-glutamate carrier, leading to two different age-dependent clinical phenotypes, neonatal intrahepatic cholestasis (NICCD, MIM#605814) and adult-onset type II citrullinemia (CTLN2, MIM#603471). Severe intrahepatic cholestasis with fatty liver is the most common presenting feature in NICCD; however, neonatal hepatitis, positive newborn screening for galactosemia, tyrosinemia, hemolytic anemia, and bleeding tendencies have been described. Here we report the first case of a European-American infant with citrin deficiency and an atypical clinical presentation. The proband was born to non-consanguineous parents and had a normal newborn screening. There was no evidence of hyperbilirrubinemia or intrahepatic cholestasis in the neonatal period. She presented with a gastrointestinal bleed at six months of age. At nine months she had another bleeding episode, and in addition significant failure to thrive, and macrocytic anemia were noted. The evaluation for her bleeding diathesis revealed an abnormal clotting profile with no hepatocellular damage. An extensive diagnostic work-up for failure to thrive included plasma amino acids with hyperglutaminemia and no elevation of citrulline levels. Her ammonia level was normal. Repeat plasma amino acid analysis exhibited a profile with elevations in citrulline, methionine and threonine. Western blotting demonstrated citrin deficiency and the patient was found to be homozygous for a deletion of exon 3 in the *SLC25A13* gene. The patient was given a high protein, low carbohydrate formula and the failure to thrive and bleeding diathesis resolved. This is the first report of citrin deficiency in a Caucasian infant. The later age at presentation of this patient without obvious evidence of intrahepatic cholestasis expands the clinical spectrum of citrin deficiency and shows that a normal newborn screening or normal chromatogram does not exclude the diagnosis of this condition.

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Homozygosity for TNSALP Mutation C1348T (Arg433Cys) Causes Infantile Hypophosphatasia Manifesting Transient Disease Correction and Variably Lethal Outcome in a Black Kindred. *M.P. Whyte[1,2], K. Essmyer[1], M. Geier[2], S. Mumm[1,2].* 1) Ctr Metab Bone Dis & Mol Res, Shriners Hospital; St Louis, MO; 2) Division of Bone and Mineral Diseases, Wash Univ Schl Medicine; St. Louis, MO.

Hypophosphatasia (HPP) features low circulating alkaline phosphatase (ALP) activity (hypophosphatasemia) from deactivating mutation of the gene encoding the tissue nonspecific isoenzyme of ALP (TNSALP) and is associated with a remarkable range of disease severity. Extracellular accumulation of inorganic pyrophosphate (PPi), one of several TNSALP substrates and an inhibitor of hydroxyapatite crystal nucleation, impairs skeletal mineralization causing rickets or osteomalacia. There is no established medical therapy. Here, we characterize HPP prevalence in blacks and determine the molecular basis for variably lethal and transiently reversible infantile HPP in a black kindred.

Our report (J Peds 108:82, 1986) details temporary correction of severe HPP in this black family where infantile HPP was fatal in two of three patients representing two sibships. At that time, the improvement in one patient followed efforts to increase TNSALP activity endogenously and suggested upregulation of the *TNSALP* gene. Here, we assessed 29 years experience to document HPPs prevalence in blacks and identified this kindreds *TNSALP* defect.

Ethnicity was known for 119 HPP families studied directly and race was ascertained for an additional 159 of 235 consultand HPP families worldwide. Only this family was black. Homozygosity for *TNSALP* missense mutation C1384T(Arg433Cys) accounted for their infantile HPP. The *TNSALP* promoter was intact. Modeling of TNSALP(433Cys) indicated compromise of the enzymes catalytic site.

We find that HPP is especially rare in blacks. Homozygous *TNSALP*(C1348T) causes variably lethal and transiently reversible HPP in this black kindred. The mutation has been reported to cause severe HPP in whites. Although not fully understood, HPP manifestations from homozygous TNSALP(433Cys) can correct, perhaps with clues for HPP's phenotypic variation and treatment.

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Peptiduria in autism and related disorders: An exploratory study. *S.G. Kahler[1,2], E. Cooper[2], D. Gaylor[3].* 1) Little Rock, AR; 2) Murdoch Childrens Research Institute Parkville, VIC, Australia; 3) Gaylor and Associates Eureka Springs, AR.

Excessive amounts of small peptides, some with opioid activity, derived from gluten and casein, have been found in the urine of many autistic children. Identification has been made by co-chromatography with synthetic standards, and immunological techniques. The peaks attributed to casein and gluten disappear with dietary elimination of their sources, and there is often amelioration of autistic symptoms. We undertook to replicate work by Reichelt, Cade, Friedman, Shattock, and others, toward the goal of confirming the identity of the peptides by mass spectrometry (MS), and quantifying them. Urine samples from children ages 1-12 years old (diagnosed with autism (N=121), developmental or intellectual delay (89), speech delay (30)), and healthy children ages 3-12 (39) were eluted with TFA and acetonitrile by HPLC from a C-18 reverse-phase column, and analyzed with dual UV detectors at 215 and 280nM. Peptiduria was quantitated as the total area under the curve (AUC) of all peaks after hippurate (~30 min) till the end of the peptide region (~68min). Retention time was expressed in relation to hippurate. Twenty-six peaks were recognized. Putative identifications include cis-indolylacryloylglycine (cis-IAG), casomorphin (CM) A5, beta(b)CM, bCM 1-4 amide, trans-IAG, alpha-gliadin, bCM 1-7, bCM 1-8. The identification of bCM 1-4 was confirmed by MS. There was notable skewing of the data from the patient groups because of high values and outliers. Statistical analysis after log transformation showed autistic children had higher total AUC ($p < 0.001$, Welchs modified t-test). Comparison of the frequency distributions showed autistic children had a significantly greater proportion of samples with values above the 75th centile for tIAG and bCM1-4 amide. Increased amounts of peptides in the urine of some children with autism/developmental problems may reflect altered intestinal function, shared etiopathogenesis, or perhaps dietary differences. Further characterization of the peaks and their significance is needed. Supported by the Murdoch Childrens Research Institute.

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DNA Fragmentation And Cell Death in Activated Lymphocytes in Wilson Disease is Triggered by Fas Antigen Engagement. *R. Prasad[1], S. Attri[1], N. Sharma[1], B.R. Thapa[2].* 1) Department of Biochemistry, PGIMER, Chandigarh, India; 2) Department of Gastroenterology, PGIMER, Chandigarh, India.

Wilson disease (WD) is an autosomal recessive disorder due to the defect in CuATP7B gene and characterized by accumulation of excess copper especially in liver and brain. Hepatocyte damage in acute WD involves CD95/Fas mediated apoptosis upregulated by oxidative stress due to copper overload. Disorders of hematopoietic system also occur in WD and one third of the patients present with lymphopenia. As activated lymphocytes express CD95/Fas antigen, CD95 mediated apoptosis due to excess copper as a cause for lymphopenia was investigated in the present study. Peripheral blood lymphocytes (PBLs) were isolated from WD patients (n=6) with hemolytic anemia as well as from age matched healthy controls (n=6). Analysis of apoptosis was carried out by DNA fragmentation, FACS analysis, Annexin-V FITC and CD95 immunofluoresence studies. DNA content and cytolytic activity of anti-Fas Ab were also investigated. Copper content in PBLs from WD patients was found to be significantly higher (0.53 0.11 nmol/mg protein) as compared to controls (0.030.02 nmol/mg protein). Characteristic DNA fragmentation in PBLs of WD patients was observed on 2.0% agarose. Lymphocytes from WD patients that were positively stained by annexin were considered as early apoptotic cells and those stained by annexin and PI both were considered as late apoptotic. No annexin staining was detected in PBLs from healthy controls. The cells with DNA content less than G1 cellular DNA content indicated any possible loss due to DNA fragmentation during apoptosis and were considered as sub G1 population. PBLs from healthy subjects showed 100% DNA content in G1 phase whereas subG1 DNA content was observed in 19+4% cells from WD patients. Percentage viability was considerably low in WD patients (79.4+4.1) as compared to PBLs from controls (99.4+0.5) and was further decreased drastically on stimulation with monoclonal anti Fas antibody (59.2+6.3) indicating involvement of CD95 mediated apoptosis which was further confirmed by detection of CD95 expression in lymphocytes from WD patients by immunofluoresence staining.

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Screening Newborns for Galactosemia Using Breath Tests of Total Body Galactose Oxidation to CO2. *D. Barbouth, H. Travers, H. Klapper, J.D. Wilkinson, V. Carver, L.J Elsas.* The Dr. John T. MacDonald Foundation Center for Medical Genetics and the Depts of Pediatrics and Epi & Public Health, Miller School of Medicine, Univ of Miami, Miami, Florida.

Expanded newborn screening is a developing public health project for an increasing number of inherited disorders of catabolism of fatty acids, amino acids and monosaccharides. Many of these disorders are lethal within days after birth, and these public health programs would benefit by in hospital point-of-care screening. Disorders of galactose-1-P-uridyl transferase (GALT) are exemplary of this problem. In this abstract we compare total body oxidation of 13C-D-galactose to expired 13CO2 in normal newborns and changes with increasing age in galactosemic children. Following parental informed consent, newborns were tested for 13CO2 enrichment of expired air before and after oral administration of 7 mg/kg of 13C-D-galactose. The ratios of 13CO2 to 12CO2 were quantitated by a dual isotope gas mass spectrometer at 0, 30, 45, 90, 100, 110 and 120 minutes and expressed as the cumulative percent dose recovered (CUMPD) at 120 minutes. Seven normal newborns were sequentially tested from 2 hours to 2 months of age. Normal newborns at 2, 24 and 48 hours of age had mean CUMPD of 3.913.62, 5.06 2.5, and 4.62 2.47 respectively (p= NS). Their mean CUMPD values at 2 months of age had increased to 13.26 2.96 (p 0.01). Four older children aged 11 days to 36 months with galactosemia and genotypes of K285N/delta5kb; Q188R/Q188R; Q188R/Q188R and N314D-R204X/K285N had CUMPD of 0.17; 0.80; 1.07 and 0.34 percent respectively. The mean CUMPD for these infants compared to normal infants at 2 months approached significance (p=0.08) but was limited by very small sample sizes. From these data we conclude that total body galactose oxidation to CO2 in breath increases at least three fold in normal newborns between 2 and 30 days of life; that galactosemic children have significantly less oxidative capacity than normal newborns and that newborn breath testing before discharge from the neonatal nursery may be an effective tool for prediction and prevention of premature morbidity and mortality due to classic galactosemia.

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Use and pitfalls of acylcarnitine analysis in diagnosis of glutaric aciduria type I. *S.E. McCandless[1], P.E. Minkler[2], M.K. Stoll[2], C.H. Hoppel[2,3].* 1) Genetics and Pediatrics/Univ Hosp Cleveland, Case Western Reserve Univ, Cleveland, OH; 2) Medical Research Service, Louis Stokes Department of Veterans Affairs Medical Center, Cleveland, OH; 3) Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

Glutaric aciduria type I (GA-I)is a severe inborn error of metabolism that often presents with severe central nervous system injury associated with metabolic stress and fever. Prior to this presentation, children may appear well, but have macrocephaly and brain MRI abnormalities. Recently, the disorder has been identified by tandem mass spectrometry newborn screening. Diagnosis is complicated by the fact that some affected individuals excrete only modest amounts of glutaric acid and 3-hydroxyglutaric acid in the urine. Our laboratory uses HPLC/MS to measure glutaroylcarnitine (C5DC) in plasma, urine and tissues. Recently, we synthesized C5DC, standardized solutions of which are used to generate multiple point standard curves (with nonanoylcarnitine as the internal standard) allowing for accurate measurement of C5DC concentrations in patient samples. Plasma (N=958) and urine (N=494) samples have been evaluated in a variety of clinically submitted samples. Plasma C5DC concentrations range from 0 to 9.6 nmol/ml, with overlap between known affected individuals and individuals with end-stage renal disease (ESRD). Also, some individuals with methylmalonic (MMA) and propionic acidemia (PA) treated with L-carnitine had plasma C5DC concentrations that overlapped the range seen in GA-I affected individuals. In urine, as has been suggested by others recently, there was a much broader range seen (0 to 3517 nm/mg creatinine), but no overlap of known affected individuals with samples from patients with MMA or PA treated with L-carnitine. As expected, C5DC excretion in urine was not elevated in ESRD. These findings suggest that plasma acylcarnitine analysis alone may lead to confusion in ESRD and in children on carnitine supplementation for other reasons. The use of urinary acylcarnitine analysis appears to clarify the diagnosis of glutaric aciduria type I, and overcomes some of these potential pitfalls.

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Differential Expression Profiles in Purine Nucleoside Phosphorylase (PNP) Deficient Mice. *F.F. Snyder, B. Danielson, H. Zhang.* Dept Medical Genetics, Univ Calgary, Calgary, Canada.

PNP deficiency is associated with cellular immunodeficiency and variable neurological involvement. We produced the NPG, Trp16Arg point mutation in the mouse which exhibits age dependent depletion of thymocyte numbers and arrested thymocyte differentiation. We have backcrossed this mutant allele 14 generations on the C57BL/6J (B6-NPG) and DBA/2J (D2-NPG) backgrounds respectively as a strategy for identifying genes which modify the phenotype. Labeled thymocyte mRNA from C57BL/6J (B6) and B6-NPG mice were hybridized against a 17K 70-mer oligo array obtained from the Southern Alberta Microarray Facility. Only 6 genes showed a greater than 2-fold increase in mutant thymocytes and none were decreased. Of these, 4 were immunoglobulin genes and 2 of lymphocyte antigen 6 complexes. Real time PCR quantitation gave a mean 42-fold increased expression for IgG2a in B6-NPG thymocytes compared to B6, whereas S100a9 showing variable expression in the array was 2.7-fold increased. The relative expression of IgG2a was increased 13-, 21- and 36-fold at 1, 2 and 3 months respectively in B6-NPG mice relative to control, thereby providing the earliest signal of the anticipated changes in the thymocyte profile. We have described a secondary deficiency of dGuo kinase in PNP deficient mice. Real time PCR showed no evidence for a reduction in transcripts in B6-NPG thymocytes or spleen relative to control. Additional genes showing variable expression are being examined. A single report indicated increased IL-18 expression in a PNP deficient child. We found serum IL-18 to be marginally increased in D2-NPG, 18316, versus D2, 15111 pg/ul. On the B6 background, however, there is a marked difference in serum IL-18 between B6-NPG, 790815, and B6, 27776 pg/ul. The broad range in B6-NPG mice being due to some mice having a greater than 8-fold increase compared to wild type. Real time PCR did not show evidence of increased expression of IL-18 in spleen leukocytes. These studies are forming the basis for a more complete understanding of early events associated with disruption of T cell differentiation in PNP deficiency and secondary sequelae. (supported by the CIHR).

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Consequences of missense in alanine:glyoxylate aminotransferase and the effect of added pyridoxal phosphate.

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Primary hyperoxaluria type 1 (PH1) results from a deficiency of liver peroxisomal alanine:glyoxylate aminotransferase (AGT) and is characterized by progressive deposition of calcium oxalate and progressive renal failure. More than 50 PH1 mutations have been documented in the AGT gene of which about 45% are missense. A consequence of missense common to many genetic diseases is mis-folding and subsequent proteasome-mediated destruction through cellular quality control surveillance. We have utilized pET vector-mediated overexpression of human mutant AGT proteins in E. coli to determine the effects of missense on enzymatic activity in isolation from eukaryotic quality control processes. For some mutants, re-folding the overexpressed enzyme in the presence of pyridoxal phosphate (PLP), an essential co-factor for the enzyme, restores measurable enzymatic activity to the protein. We have also used in vitro transcription/translation with rabbit reticulocyte lysates to investigate the post-translational stability of mutant AGT protein in a eukaryotic milieu. Most mutants studied showed varying degrees of instability in this system but were stabilized in the presence of PLP. These mutants were predominantly the ones for which enzymatic activity could be recovered after expression in E. coli and re-folding. Our results suggest that PLP can function as a chaperone for certain mutant proteins, restoring enzymatic activity and stability, and that, under optimal conditions, mutant AGTs may have biological activity. This work was funded by a grant from the Garrod Association of Canada and the Hospital for Sick Children Foundation, Canada.

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Gene expression profiling in prosaposin deficient mice: molecular alterations precede neuronal pathology. *Y. Sun[1], L. Jia[1], M.T. Williams[2], C.V. Vorhees[2], D.P. Witte[3], G.A. Grabowski[1].* 1) The Division and Program in Human Genetics; 2) Division of Neurology; 3) Division of Pediatric Pathology, Cincinnati Childrens Hospital Medical Center, Cincinnati, Ohio.

Prosaposin encodes, in tandem, four small acidic activator proteins (saposins) with specificities for glycosphingolipids (GSL) hydrolases in lysosome. The critical roles for saposins in GSL metabolism are highlighted by the extensive GSL storage in various CNS areas in the human and mouse prosaposin deficiencies. Our hypomorphic prosaposin deficient mouse model, PS-NA, exhibit 45% of wild type expression levels of saposins in the brain. PS-NA mice survived up to 220 days and showed neurological pathology that includs GSL storage in neurons and loss of Purkinje cells. The predominantly accumulated lipids are lactosylceramide (LacCer) and glucosylceramide (GC). Impairment of neuronal function was observed by 6 weeks using narrow bridge test. To explore the molecular mechanism(s) of disease progression, whole transcriptome microarray analyses of brain tissues were conducted with mRNA from PS-NA and control mice at birth, 4, 12 and 18 weeks. The expression of the proinflammatory genes were increased by age of 4 week. The genes involved in cell death pathway were differentially expressed with response early in cerebrum and delayed in cerebellum. Immunohistochemistry demonstrated activation of microglia cells and astrocytes, along with neuronal degeneration after 12 weeks. These results show that regionally specific gene expression abnormalities preceded the histological changes and neuronal pathology in the PS-NA mouse. The proinflammatory responses in microglial cells and astrocytes might propagate the disease progression. Temporal profiling of gene expression changes provides insight for defining molecular mechanism in the progression of GSL storage disease.

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Correlation between Interleukin-6 promoter gene polymorphism and the Mainz Severity Score Index for Fabry disease. *D. Elstein[1], C. Wybra[2], M. Beck[2], G. Altarescu[1].* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) University of Meinz, Germany.

Objective: To determine the functional interleukin-6 (IL-6) -174 GC allelic variants in relation to disease severity markers using the Mainz Severity Score Index (MSSI) in a cohort of patients with Fabry disease. Methods: 56 patients (34 hemizygous males, 22 females) including five children (one boy) were genotyped for the IL-6 -174 GC polymorphism. Results were correlated with the MSSI that included a general, neurological, cardiac, and kidney sub-scores and a composite total score. Results: Mean age of the adults was 42 range: 26-58) years with 30 patients having received enzyme replacement therapy (ERT) for a mean of 3.1 (2.2-4.0) years. Mean total MSSI was 26.7 (14.2-39.2) points. Prevalence of allelic variants of the IL-6 polymorphism, 48%G/G (wild type), 27%G/C, and 25%C/C, is significantly different ($p<0.05$) from the distribution reported among Caucasians, but C allele frequency was similar to that reported among patients with lacunar strokes. A statistically significant correlation was found between the neurological MSSI sub-score and the C/C genotype ($p=0.001$), with MSSI sub-scores approximately two-fold higher among patients with the C/C genotype, although the majority (12/14 patients) of those with the C/C genotype had had benefit of ERT. Conclusions: The prevalence of the C/C genotype of the IL-6 promoter polymorphism among patients with Fabry disease is greater than in control Caucasian populations, and appears to carry a greater risk for developing stroke and other neurological manifestations. Since the pro-inflammatory cytokine IL-6 may be released shortly after hypoperfusion of the brain, the interesting question raised by the current study is whether the C/C genotype may be a modifier and/or marker of microvascular abnormalities in Fabry disease.

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Visceral variant of Niemann-Pick disease type C1 in an adult. Report of the third case. *L. Dvorakova, M. Bouckova, J. Sikora, M. Hrebicek, L. Stolnaja, H. Hulkova, M. Elleder.* Inst Inherit Metabol Disorders, Charles Univ, 1st Sch Medicine, Prague 2, Czech Republic.

Accidental autopsy findings of hepatosplenomegaly, lymphadenopathy and histopathological signs of visceral lysosomal storage in a 53 year old female with pulmonary embolism raised the initial suspicion of Gaucher disease (GD). There was no prior significant medical history. Additional histopathological evaluation excluded GD and rather supported the diagnosis of visceral form of acid sphingomyelinase deficiency (Niemann-Pick disease type B - NPB) or much rarer disorder, variant adult visceral form of Niemann-Pick disease type C. To verify the histopathological findings, we analyzed potentially involved genes. As only formalin fixed paraffin embedded (FFPE) tissues from the proband were available, we examined probands living relatives (mother and two sons). No pathogenic sequence variation in SMPD1 gene, mutated in NPB, was identified in any of the relatives examined. Consequently we analyzed genes associated with Niemann-Pick disease type C. While no mutations were detected in NPC2 (HE1), a heterozygous substitution c.1997GA was identified in exon 13 of NPC1 gene of all three relatives. Analysis of DNA isolated from FFPE tissues (spleen and lymph node) showed no variation in NPC2, while the presence of c.1997GA in NPC1 was confirmed in both tissues. In addition, heterozygous substitution c.2882AG was found in exon 19 of NPC1 gene. Both variations c.1997GA (S666N) and c.2882AG (N961S) detected in NPC1 gene are novel. The PCR/RFLP analysis of 318 control alleles excluded the possibility that the identified variations are common polymorphism. The expected pathogenicity of both variations is supported by their location in regions important for the protein function. The results support the diagnosis of extremely rare adult visceral form of Niemann-Pick disease type C othervise dominated by neurovisceral phenotype. Despite the low number of patients reported (two), this form of NPC should be considered in differential diagnosis of isolated hepatosplenomegaly with foam cells in the adult age. *This work was supported by grants IGA-MZ CR 8351-3 and VZ MSM CR 20620806*.

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Clinical and genetic characterization of central nervous system folate deficiency. *F. Scaglia[1], P. Moretti[1,2], S. Peters[3], D. Del Gaudio[1], K. Hyland[4], T. Bottiglieri[4], R. Hopkin[5], E. Peach[5], B. Roa[1], C. Bacino[1].* 1) Dept Mol & Human Genetics; 2) Neurology; 3) Pediatrics, Baylor Col Medicine, Houston, TX; 4) Institute of Metabolic Disease, Baylor University Medical Center, Dallas, TX; 5) Human Genetics, Cincinnati Childrens Hosp Med Ctr, Cincinnati, OH.

We studied the clinical and biochemical features of 5 girls and 2 boys with an isolated deficiency of folate in the central nervous system (CNS). All patients had low cerebrospinal fluid (CSF) levels of 5-methyltetrahydrofolate, the biologically active form of folate in CSF and blood. Red blood cell folate, serum folate, and total plasma homocysteine were normal. All children exhibited psychomotor retardation, developmental regression, seizures and diskinesia with variable response to folinic acid. Two females had manifestations evocative of Angelman syndrome and a third exhibited features of Rett syndrome. Angelman syndrome DNA methylation studies and *UBE3A* mutation analysis were normal. No *MECP2* mutations or deletions were found. Five of seven patients demonstrated considerable repetitive behaviors, decreased eye gaze, and communication deficits that warranted a formal evaluation for autism. Using the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview - Revised, three subjects met criteria for autism, demonstrating deficits in communication and socialization that mirror those observed in children with idiopathic autism. Formal evaluations are currently being completed with two other patients. The selective CNS folate deficit suggested the presence of defective folate transfer across the blood brain barrier. We sequenced genes encoding proteins involved in the transport of folate across mammalian cell membranes (*RFC1*, *FBP1*, *FBP2*, *FBP3*), methylenetetrahydrofolate reductase (*MTHFR*) and dihydrofolate reductase (*DHFR*). We found no mutations in the coding regions of these genes. These findings demonstrate that children with autism, developmental delay, regression, and seizures may have abnormalities of CNS folate. Future studies will determine the general relevance and frequency of these findings and elucidate the molecular and biochemical mechanisms of reduced CSF folate in these patients.

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The natural history of cognitive abilities in Hurler-Scheie syndrome: Eight years longitudinal evaluation of three siblings treated with recombinant human -L-iduronidase (laronidase, Aldurazyme). *K. Bjoraker, L. Charnas, C.B. Whitley, K. Delaney, E.G. Shapiro.* Dept Pediatrics, Univ Minnesota, Minneapolis, MN.

The natural history of cognitive development and CNS function in the intermediate form of MPS I, Hurler-Scheie syndrome, is unknown. Learning disabilities have been described; no decline was presumed to occur as in Hurler syndrome. Neuropsychological testing is not routine; thus no data are available. Are the cognitive effects are secondary to health factors (e.g., limited mobility, sleep apnea, hearing loss, hydrocephalus, poor motor skills), or are they directly related to neurotoxic effects of enzyme deficiency. IV administration of recombinant enzyme (ERT) in the canine model does not increase enzyme activity across the blood-brain barrier. ERT has improved the somatic features and corrected secondary effects on the nervous system. This allows assessment of whether cognitive function is stable in enzyme treated patients or if impairments are a direct neurotoxic effect of enzyme deficiency. Method: 3 siblings with MPS IH-S received serial neuropsychological, cranial MRI and neurological assessments over 8 years. The 15 year old received ERT for 7 years; the two siblings for 4. All had increased mobility and range of motion. All had early carpal tunnel release. The 12 and 14 year old received VP shunts for hydrocephalus prior to ERT. The eldest required VP shunt 4 years after ERT initiation. Results: IQ decreased over time in all 3 children. Memory encoding showed a dramatic decline beginning at age 12 in the 14 and 15 year olds. Math ability, visual motor skills, and spatial perception have significantly declined. Attention span, executive functions and reading ability are not affected. Adaptive skills are normal. Cranial MRI is unchanged. Conclusions: Progressive slowing of cognitive development with particular impairment in memory encoding occurs in adolescents with MPS I H-S receiving ERT. The declines are likely a primary effect on the brain and not secondary effects of MPSI on the nervous system. Additional study is needed to determine if late decline is a common feature of MPS I H-S.

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Autism as the major presenting feature of inborn errors of metabolism. *Y. Choy[1], L. Ngu[1], W. Keng[1], B. Chen[2], Y. Zabedah[3], A.K.D. Pertiwi[3].* 1) Genetics Unit, Kuala Lumpur Hosp/Ped Inst, Kuala Lumpur, Malaysia; 2) Metabolic Laboratory, Kuala Lumpur Hospital; 3) Metabolic Laboratory, Institute of Medical Research.

Autism is a common childhood disorder and majority of the patients have no known underlying cause. In 5 to 10% of the patients, there is an identifiable cause including Fragile-X syndrome, Rett syndrome, tuberous sclerosis and chromosomal abnormalities such as duplication 15q11q15. Inborn errors of metabolism (IEM) in which autism has been reported as the main presenting feature include untreated phenylketonuria, urea cycle defects, mitochondrial dysfunction, purine and pyrimidine defects. Over the past 5 years, there were 196 patients referred to our center for further evaluation of autism. They had no neuroregression or physical handicaps, birth defects, neurocutaneous stigmata or specific features to suggest IEM such as cyclical vomiting, recurrent episodes of confusion or lethargy. The standard evaluation protocol included high resolution karyotype, Fragile-X testing, MECP2 testing, MRI of the brain, lead level and metabolic screen including lactate/pyruvate, ammonia, uric acid, amino acid, organic acid and acylcarnitine.

We had identified 5 cases of IEM (2.5%) in this cohort of patients. One of them had mild chronic variant of maple syrup urine disease (MSUD), another patient had non-ketotic hyperglycinemia. One had glutaric acidemia type I and one had mitochondrial dysfunction with persistent lactic acidosis. One had hypouricosuria probably due to cytosolic 5'nucleotidase superactivity. They were all treated and showed variable improvement. The most remarkable improvement was observed in the 5-year-old boy with MSUD. He actually improved with a lower protein diet when his mother stopped his milk to limit intake of casein. The findings of these patients may argue for metabolic investigation in patients with autism. Indeed, the parents of the boy with MSUD questioned the delay in metabolic testing. The diagnosis in these patients could be missed by newborn screening as they were milder variant of the disorders.

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Interactions between variants of the endothelial nitric oxide synthase gene and dietary intakes of vitamin C, fruit and vegetable in association with plasma markers of endothelial function and inflammation among US diabetic men. *C. Zhang, F. Hu.* Nutrition, Harvard School of Public Healt, Boston, MA.

Background: Endothelial dysfunction and vascular inflammation contribute substantially to the accelerated atherogenesis associated with type 2 diabetes. Associations have been described between two potentially functional variants of the endothelial nitric oxide synthase (ENOS) gene (T(-786)C and G894T) and makers of endothelial function in non-diabetic individuals. Dietary vitamin C can have antioxidant-mediated tempering influence on endothelial function and inflammation in vivo. Population: 641 men (97% Caucasian) had confirmed type 2 diabetes among 18,159 men in the Health Professionals Follow-up Study. Results: After adjustment for age, body mass index, duration of diabetes and other covariates, plasma levels of soluble vascular cell adhesion molecule (sVCAM-1), soluble intercellular cell adhesion molecule (sICAM-1) and tumor necrosis factor alpha (TNF-) were significantly higher in men with the 894 G/T or T/T genotype than those with the 894G/G genotype (adjusted mean 1385 vs. 1299 ng/ml, P=0.002 for VCAM; 366 vs. 348 ng/ml, P=0.02 for ICAM; 2984 vs 2847 pg/ml, P=0.01 for TNF-). (-786) G/T or T/T genotype was associated with significantly elevated levels of sVCAM and TNF- as well. In multivariate linear regression models, ICAM-1 and VCAM levels were associated with significant interactions between the G894T variant and dietary intake of vitamin C and fruit and vegetable as continuous variables (all P-values for interaction <0.01), respectively. By using 2 categories of vitamin C and fruit and vegetable (dichotomized by median), we found significantly different effects of the G894T genotypes on ICAM-1 and VCAM levels across these dietary variables; significant elevations of ICAM-1 and VCAM levels were associated with the G894T variant only in men with lower intakes of vitamin C (<154mg/day) or fruits and vegetables (<5 servings/day. Conclusions: Our study suggests that effects of the functional variant of the ENOS gene on CAM levels were modified by dietary intake of vitamin C and fruits and vegetables- the major source of dietary antioxidants.

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FREQUENCY OF A1298C (MTHFR) POLYMORPHISM IN PATIENTS WITH CORONARY ARTERY DISEASE IN NORTHWEST MEXICAN POPULATION.

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INTRODUCTION. The coronary artery disease (CAD) is the most important cardiovascular disorder and the principal world cause of death. It has been considered multifactorial disease because of different risk factors (genetics and environmental). Between genetics factors we can find modifications in methylenetetrahydrofolate reductase (MTHFR) levels. This enzyme plays a role in processing amino acids like homocysteine, which is cause of damaged in vascular endothelium with expresion of aterogenic factors. Some studies have described an association between A1298C (MTHFR) polymorphism and CAD, although this association is contradictory in anothers studies and its association is unknown in northwest Mexican population. METHODS. In this study, genomic DNA from 67 CAD patients (abnormal coronariography) and 58 persons without CAD and background of CAD, diabetes mellitus and arterial hypertension were genotyped. We analized the allelic and genotypic distribution in A1298C polymorphism. RESULTS. The genotypic frecuency of 1298AA was 56.72% in CAD patients and 48.27% in control group; 1298CC was 13.43% in CAD patients and 15.52% in control group, whereas 29.85% in CAD patients and 36.21% in control group were heterozygous. Statistical differences was not observed between groups ($p<0.05$). CONCLUSIONS. Association was not observed between the genotypes and CAD, showing that this polymorphism possibly not play a role as risk factor of CAD.

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Differentiation of unexplained hypouricemia. *I. Sebesta[1,2], B. Stiburkova[2], J. Krijt[2], O. Martincova[2], E. Hruba[2].* 1) Institute of Clinical Biochemistry and Laboratory Diagnostics; 2) Institute of Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, Prague, Czech Republic.

Decreased serum uric acid levels result from decreased production and/or renal urate wasting. Hypouricemia is defined as a serum urate levels less than 2 mg/dL(119 umol/L). Primary hypouricemia is caused by several purine metabolic disorders. Recent identification of the renal urate transporter (URAT1,encoded by SLC22A12) led to the molecular elucidation of idiopathic renal hypouricemia (MIM 220150), which is a predisposition towards exercise-induced acute renal failure. Secondary hypouricemia in different disorders is related to proximal tubular damage, use of uricosuric drugs etc. Hypouricemia is sometimes overlooked. Therefore we have set up the diagnostic guideline for differential diagnosis. Uric acid was quantified by specific enzymic method and red cell enzyme with urinary purine nucleosides were measured by methods adapted to HPLC. Results: A proposed scheme for the investigation of unexplained hypouricemia is as follows. Estimation of: 1) excretion fraction of uric acid , 2) urinary xanthine, S-sulphocysteine, thiosulfate, 3) measurement of (deoxy)guanosine,(deoxy)inosine in urine, if positive - assay of purine nucleoside phosphorylase in erythrocytes, 4) analysis of URAT 1 gene. The evaluation of clinical history with attention to renal failure, urolithiasis, seizures and immunodeficiency is indispensable. This flow chart allows to differentiate a) idiopathic renal hypouricaemia, b) hereditary xanthinuria, c) molybdenum cofactor deficiency, d) purine nucleoside phosphorylase deficiency. Moreover, primary hyperuricemia can be excluded. Using this flow chart we have detected four new families of Czech origin with hereditary xanthinuria, one case of hereditary renal hypouricaemia and one patient with molybdenum cofactor deficiency from 3,400 serum and urine samples received during last three years. In conclusion-the exclusion of primary hypouricemia allows to search for the other causes. Accessible guideline will help for early diagnosis of purine disorders associated with hypouricemia.(Supported by grants-MSM 0021620806, VZ 64165,CZ).

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Molecular analysis of glycogen storage disease type Ia, Ib, and III in Korean patients. *J. Yang[1], J. Lee[2], J. Kim[1], S. Park[1], G. Kim[1,2], J. Choi[2], H. Yoo[1,2].* 1) Genome Research Ctr, Asan Medical Center, Seoul, Korea; 2) Medical Genetics Clinic & Laboratory, Asan Medical Ctr, Seoul, Korea.

Glycogen storage disease (GSD) Ia, Ib, and III are rare autosomal recessive disorders. Their clinical manifestations such as recurrent hypoglycemia, hepatomegaly, growth failure, hyperlipoidemia, and lactic academia are so mimicking that it is often difficult to differentiate each other. GSD Ib is distinguished from GSD Ia and III by the presence of persistent neutropenia. The affected genes of GSD Ia, Ib, and III are glucose 6-phosphatase, catalytic (G6PC), glucose 6-phosphate transporter (G6PT1 or SLC37A4), and amylo-1,6-glucosidase (AGL), respectively. We studied 17 unrelated Korean patients with GSD Ia, Ib, and III. The G6PC gene was initially analyzed in the patients without neutropenia. The patients with neutropenia were analyzed for the presence of mutation in the G6PT1. Subsequently, AGL was analyzed in patients without mutation in G6PC and G6PT1 genes. Out of 17 patients clinically diagnosed as GSD, we identified 12 of GSD Ia, 2 of GSD Ib, and 3 of GSD III patients. We found 10 different mutations; three mutations (p.F51S, p.G122D, and c.624 G>T) in G6PC, three mutations (p.W138R, p.P191L, and p.L328VfsX52) in G6PT1, and four mutations (pR428K, p.L1139P, c.1735+1 G>T, and p.S603PfsX6) in AGL. Among these mutations, six were novel mutations. Those were p.F51S in G6PC, p.W138R; p.L328VfsX52 in G6PT1, and p.L1139P; c.1735+1 C>T; and p.S603PfsX6 in AGL. The mutations of three genes were heterogeneous except G6PC where the mutation was homogeneous, showing a hot spot, c.648 G>T (91.7% of allele frequency). In conclusion, we demonstrated a strategy of molecular analysis for type Ia, Ib, and III glycogen storage diseases that share common clinical findings. According to this strategy, we identified ten different mutations including six novel mutations in three genes involving glycogen storage disease Ia, Ib, and III.

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Direct screening of R83C mutation in Tunisia as a rapid non-invasive diagnosis tool in south Mediterranean populations. *W. Cherif[1], E. Barkaoui[2], N. Tebib[2], Ch. Charfeddine[1], S. Chakroun[1], S. Abdelhak[1], MF. Ben dridi[2].* 1) Molecular investigation of Gen, Institut Pasteur , Tunis, Tunis, Tunisia; 2) service de pédiatrie du CHU la Rabta Tunis, Tunisia.

Glycogenosis Type Ia (GSD Ia) is an inherited metabolic disorder caused by the deficient of D-glucose-6-phosphatase (G6Pase) activity. This disease is an autosomal recessive disorder and it is pan-ethnic , it occurs in 1 / 100000 to 1/300000 individuals. GSD Ia is characterized by hypoglycemia, lactic acidemia, hepatomegaly, hyperuricemia, hyperlipidemia and renal dysfunction leading to chronic renal failure as a late manifestation of the disease. The confirmation of the clinical diagnosis is usually performed by liver biopsy .The molecular investigation may provide a time and cost effective and less invasive tool for diagnosis of GSD Ia . Recently, by the study of the molecular basis of GSD Ia in Tunisian patients we have shown that R83C is relatively frequent. Mutation screening have shown that 6 out of 9 patients were homozygous for R83C. We propose here the use of direct screening of this mutation for rapid diagnosis of GSD Ia. Five patients presenting with clinical features leading to a suspicion of GSD Ia have been explored by direct sequencing of exon 2 of G6PC. Sequence analysis revealed that the 5 patients had the R83C mutation thus confirming the clinical diagnosis and circumventing liver biopsy and enzymatic investigation. We propose the use of direct screening of R83C mutation as a rapid and valuable tool for diagnosis of GSD Ia in south Mediterranean populations.

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Increased brain guanidino compounds suggests disrupted creatine metabolism in the succinate semialdehyde dehydrogenase (SSADH) deficient mouse. *K.M. Gibson[1], E.E.W. Jansen[2], N.M. Verhoeven[2], A. Schulze[3], H. Senephansiri[4], M. Gupta[4], C. Jakobs[2].* 1) Med Genet/Peds/Pathol, Univ Pittsburgh Sch Med, Pittsburgh, PA; 2) Clin Chem, Vrije Univ Med Ctr, Amsterdam, Netherlands; 3) Div Metab Dis, Univ Child Hosp, Heidelberg, Germany; 4) Molec Med Genet, Oregon Hlth Sci Univ, Portland, OR.

Guanidinoacetate (GA), an endogenous intermediate in the conversion of L-arginine to creatine, is increased in GAMT deficiency, one of the inherited disorders of creatine biosynthesis. Conversely, guanidinobutyrate (GB), first isolated from mammalian brain in 1959, has been detected in humans only when GABA is pharmacologically elevated with vigabatrin. We previously demonstrated decreased L-arginine levels in cortex (Cx) and hippocampus (Hc) from SSADH-/- mice. Since these animals have increased brain GABA, we quantified guanidino species in brain extracts. Total homogenates revealed elevated GA and GB (SSADH+/+ (n=5) 24.6 +/- 1.1 (SE) and 7.9 +/- 0.3 nmol/100 mg protein; SSADH-/- (n=3) 97.2 +/- 9.9 and 168.5 +/- 7.1), respectively ($p<0.04$). These results were confirmed in regional (Ce-cerebellum) extracts: (Ce) GA and GB (SSADH+/+ (n=6) 31.3 +/- 1.9 and 12.5 +/- 0.3; SSADH-/- (n=7) 49.0 +/- 4.3 and 279.7 +/- 19.3); (Hc) (SSADH+/+ (n=6) 40.5 +/- 5.2 and 9.5 +/- 0.3; SSADH-/- (n=6) 62.7 +/- 7.7 and (n=7) 188.9 +/- 19.1); (Cx) (SSADH+/+ (n=6) 64.2 +/- 3.6 and 11.8 +/- 0.3; SSADH-/- (n=3) 218.7 +/- 50.0 and (n=4) 168.8 +/- 5.3), respectively. Guanidinopropionate was not elevated. Guanidino species have been shown to generate reactive oxygen intermediates, activate GABA(A) receptors, and disrupt creatine metabolism, implying new pathomechanisms in this disorder. Increased GA most likely derives from competitive inhibition of the GAMT reaction by GB. Our results suggest that SSADH deficiency is the first inborn error of metabolism with endogenously elevated GB, supported by the preliminary observation of elevated GB in the urine derived from an SSADH-deficient patient. Our findings raise the potential for disruption of the nitric oxide signaling system in this disorder.

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Identification of interaction partners of Hermansky-Pudlak syndrome proteins. *W. Westbroek[1], A. Helip Wooley[1], H. Dorward[1], M. Ayub[1], R. Hess[1], J. Tavernier[2], M. Huizing[1], WA. Gahl[1].* 1) Medical Genetics Branch, NHGRI/MGB/NIH, Bethesda, MD; 2) Department of Medical Protein Research, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium.

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder, characterized by various degrees of skin, hair and eye hypomelanosis, and a bleeding diathesis. The clinical symptoms result from defective biogenesis of lysosome-related organelles (LROs), such as melanosomes in melanocytes and dense bodies in platelets. Currently, mutations in seven genes are known to cause HPS-1 through HPS-7 in humans. However, with the exception of HPS2, the biological functions of the HPS proteins in LRO biogenesis remain elusive. It is known that different HPS proteins can interact to form Biogenesis of Lysosome-Related Organelle Complexes (BLOCs). First, we studied interactions between the human BLOC2 (HPS3, HPS5, and HPS6) and BLOC3 (HPS1 and HPS4) components by yeast two-hybrid screening. We did not detect direct interactions, which could be due to detection limitations of the yeast two-hybrid method. We then employed the innovative MAPPIT (Mammalian Protein-Protein Interaction Trap) technique to study interactions between the members of BLOC2 and BLOC3. This mammalian cytokine receptor type I based system identifies direct protein-protein interactions in their physiological context without depending on nuclear translocation of the bait and prey proteins. Interaction studies between the known members of the BLOCs are ongoing. Simultaneously, we started screening HPS1 and HPS5 against the MAPPIT placenta library to find new BLOC2 or BLOC3 members. Identification of new interaction partners of HPS proteins will not only give a better insight into the cell biology of this syndrome but will also provide strong candidate genes for patients that are clinically but not genetically diagnosed with Hermansky-Pudlak syndrome.

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P450 Oxidoreductase: The cause of Antley-Bixler syndrome and apparent combined 17- and 21-hydroxylase deficiency. *C. Shackleton[1], J. Marcos[1], H. Ivison[2], V. Dhir[2], B. Hauffa[3], E. Malunowicz[4], P. Stewart[2], W. Arlt[2].* 1) Children's Hospital Oakland Research Institute, Oakland, CA; 2) University of Birmingham, Division of Medical Sciences, Birmingham, UK; 3) University Children's Hospital, Essen, Germany; 4) Childrens Health Memorial Institute, Warsaw, Poland.

Introduction: For 30 years a variant of congenital adrenal hyperplasia (CAH) has been observed that appears as combined 17- and 21-hydroxylase deficiency (17-, 21-HD) clinically leading to ambiguous genitalia and cortisol deficiency. Separately, many patients with the craniofacial and skeletal deformity Antley-Bixler syndrome were found to have impaired steroidogenesis. The common cause was recently defined as P450 oxidoreductase deficiency (ORD). Addressing the importance of documenting new disorders we describe 13 new patients. Methods: GC/MS urine steroid panels were developed for specific diagnosis of ORD in childhood and prenatally. DNA analysis was performed on most patients. Results: *Children*: Diagnostic steroid metabolites were abnormal in all patients studied. Elevated excretions were found of: 1) corticosterone metabolites (distinctive of 17-HD), 2) 17-hydroxyprogesterone and 21-deoxycortisol metabolites (distinctive of 21-HD), and 3) metabolites of precursors pregnenolone and progesterone. Androgen metabolite excretion was low. *Prenatal diagnosis*: estriol (E3) levels were low in prenatal screening of mothers with ORD fetuses. One mother showed elevated excretions of epiallopregnanediol (EAP), a metabolite of fetal pregnenolone, and androsterone (A) associated with the fetal and maternal virilization. *Genetic analysis*: An A287P mutation was the most prevalent, affecting at least one allele in 10 cases. Homozygous A287P inevitably had bone malformation. Mutations included exonic, frameshift, splice site and missense leading to the change of a single amino acid in the OR protein. Conclusion: ORD can be diagnosed postnatally from urinary steroid ratios. Low E3 and elevated excretion of EAP and A are likely diagnostic for ORD prenatally. A287P is the most common mutation in Caucasian ORD patients. Considering cases reported since finding the cause of the disease, ORD may be second to 21-HD in CAH incidence.

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STUDY OF NATURAL HISTORY AND GENOTYPE ANALYSIS OF JUVENILE GM2 GANGLIOSIDOSIS.

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Juvenile GM2 gangliosidosis (jGM2) is a rare neurodegenerative disorder caused by lysosomal HexA deficiency.Objective:natural history and genotype-phenotype correlation study of 21 patients from 14 unrelated families:15 diagnosed with Tay-Sachs variant(TSV) and 6 with Sandhoff variant(SV).Results:The mean age of onset was 5.3 years (1.5-17years), 11 males and 10 females. The most common symptoms at onset were gait ataxia (13/21; 63%) and speech disturbances along with coordination problems (12/21; 57%).Interestingly, developmental delay were not as important being present as first symptom in only 6/21 patients(28.6%). Correlating the genotype-phenotype, three distinctive clusters were observed.In one group, patients with c.533G>A(R178H) in one of the HEXA alleles along with an infantile mutation presented with an earlier and more severe neurological presentation.In a second group, patients with c.1496G>A(R499H) along with an infantile mutation showed an intermediate phenotype.In the third group, three siblings with a combination of c.805G>A(G269S) and an infantile mutation presented the mildest phenotype.These three patients presented in the late second decade with pronounced psychiatric symptoms.A detail clinical inquiry revealed that these patients had motor and behaviour disturbances during childhood.Two new mutations were found in the HEXA gene were characterized:c.681C>G(Y227X)and c.1330+1G>A. Among those patients with SV, four non-reported mutations were identified:c.410G>A(C137Y),c.769T>G(Y266D),c.1057G.C(G353R) and 115delG with the latter being associated with a more severe phenotype.Conclusion: Recent studies have been described clinical variability among patients with this condition (Hendriksz et al2004).In the present study, we observed considerable heterogeneity in the clinical presentation as well as in the progression of jGM2.

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Two successful pregnancies with maternal diagnosis of Morquio A - clinical challenges and lessons learned. *D.J. Zand[1], D.R. Stewart[1], D. Driscoll[2], P. Kaplan[1].* 1) Division of Metabolism, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Dept. of Obstetrics, University of Pennsylvania Medical Center, Philadelphia, PA.

A 26 year old african american woman with both clinical diagnosis and biochemical confirmation of Morquio type A (mucopolysaccharidosis IV type A; OMIM #253000) was followed through two pregnacies. Both pregnacies carried concern for IUGR, and resulted in delivery of term, healthy children via cesarian section. The patient had been intermittantly lost to follow-up as a child and in-between pregnancies. At the time of her first delivery, she was wheelchair bound and required nursing assistance for daily care. Previous interventions had included cervical spine fusion and multiple surgical procedures for neurologic bowel and bladder. Pregnancy related evaluations were remarkable for previuosly undiagnosed and severe pulmonary hypertension. While both the patient and her younger sibling carried a diagnosis of Morquio type A, her sibling had declined on-going care for her medical needs and passed due to disease complications. The daily care provided to this patient probably altered the pregnancy outcomes, despite the severe limitations on uterine growth, pulmonary capacity, and cardiac function. Similar case histories could not be found in the literature for this diagnosis. With improvements in medical care for patients with Morquio type A, management for both routine health and reproductive concerns may be more common, demanding ease of coordinated care.

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Prediction of insulin phenotypes from gene expression data by using a mathematical model of the insulin signaling pathway. *G. Sriram[1,2], J.C. Liao[2], K.M. Dipple[1,3].* 1) Human Genetics, David Geffen School of Medicine at UCLA; 2) Chemical Engineering, Henry Samueli School of Engineering and Applied Science at UCLA; 3) Pediatrics, David Geffen School of Medicine at UCLA, Mattel Childrens Hospital at UCLA.

Glycerol kinase deficiency (GKD) is an inborn metabolic disorder that predisposes affected individuals to insulin resistance and diabetes mellitus. (Gaudet et al. Am J Hum Genet 66: 1558, 2000). Our previous microarray studies revealed significantly altered expression of key genes involved in the insulin signaling pathway in glycerol kinase-knockout (Gyk k/o) mice. In this study, we employed a mathematical model of the insulin signaling pathway to quantitatively understand the effects of altered gene expression on insulin sensitivity. A mathematical model of the insulin signaling pathway was developed, based on an earlier model (Sedaghat et al. Am J Physiol Endocrinol Metab 283: E1084, 2002). The model uses gene expression values as inputs, and simulates the translocation of the glucose transporter GLUT4 for a fixed dose of insulin. The extent of GLUT4 translocation is used as a measure of insulin sensitivity. The simulation results indicated that in the brown fat of Gyk k/o mice, the altered level of expression of the genes protein kinase C (PKC, overexpressed 3.0-fold relative to wild type), protein tyrosine phosphatase (PTP, overexpressed 2.8-fold), and phosphatidylinositol-3-kinase (PI3K, underexpressed 2.8-fold) contributed significantly to a reduced GLUT4 translocation. The underexpression of PKC alone contributed to a 33.7% reduction in GLUT4 translocation (for an insulin dose of 10^{-7} M for 15 min). Together, the altered expression levels of PKC, PTP and PI3K contributed to a 44.9% reduction in GLUT4 translocation (for the same insulin dose). In conclusion, mathematical modeling studies reported here quantitatively demonstrate that altered expression of genes in the insulin signaling pathway in Gyk k/o mice predict decreased insulin sensitivity in the animals. Further analysis of gene expression data in other tissues will facilitate an improved understanding of the role GKD in insulin resistance and diabetes.

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Glycerol kinase deficiency alters gene expression of genes involved in fat and carbohydrate metabolism and insulin signaling. *L. Rahib[1], N.K. MacLennan[2], S. Horvath[3,4], K.M. Dipple[1,2,3].* 1) Biomedical Engineering, UCLA, Los Angeles, CA; 2) Pediatrics, UCLA, Los Angeles, CA; 3) Human Genetics, UCLA, Los Angeles, CA; 4) Biostatistics, UCLA, Los Angeles, CA.

Glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism at the interface of glucose and fat metabolism. Individuals with a glycerol kinase (GK) mutation, N288D, are at risk for insulin resistance and diabetes mellitus (Gaudet et al., Am J Hum Genet 66:1558, 2000). Thiazolidinediones treat type 2 diabetes by inducing GK expression in adipocytes (Guan et al., Nat Med 8:1122, 2002). The purpose of this study was to evaluate the levels of expression of genes involved in carbohydrate and fat metabolism as well as insulin signaling in a glycerol kinase (Gyk) knock out (ko) mouse model. Total RNA was extracted from brown fat from three day old Gyk ko and wildtype (wt) male mice. Microarray analysis determined that there were 668 genes that were differentially expressed (1.5 fold) between ko and wt mice. This included 28 genes involved in lipid metabolism and 12 genes involved in carbohydrate metabolism. Nineteen genes involved in the insulin signaling pathway were altered in the Gyk ko male. Real Time-PCR confirmed the down-regulation of genes involved in lipid and carbohydrate metabolism including Gyk, glycogen synthase 2, alpha-N-acetylglucosaminidase, and galactose-4-epimerase as well as the upregulation of sphingosine phosphate, lipin 1, sulfotransferase family 1A, phenol-preferring member 1, and flavin containing monooxygenase 1. Genes involved in insulin signaling and insulin resistance that were downregulated and confirmed were insulin-like growth factor 1, fatty acid synthase, Jun oncogene, mitogen-activated protein kinase 6, and eukaryotic translation initiation factor 4E. The upregulation of leptin, sterol regulatory element binding factor 2, mitogen-activated protein kinase kinase kinase 6, and CCAAT/enhancer binding protein delta was confirmed. Further investigations of these genes may provide insight into the role of GK in insulin signaling, insulin resistance and type 2 diabetes mellitus.

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Congenital disorder of glycosylation type 1a in Malaysia. *M. Thong, S. Chow, J. Lee, O. Asma.* Dept Pediatrics, Univ Malaya Medical Ctr, 59100 Kuala Lumpur, Malaysia.

Reports of congenital disorders of glycosylation (CDG) are uncommon in Asian populations. We report a one-day-old Malay baby girl who was referred for dysmorphic features. The parents were second cousins with no significant family history of note. The antenatal period was uneventful and she was delivered at term via Caesarean section with a birth weight of 3.6 kg. She was found to have bilateral inverted nipples, abnormal fat distribution over her thighs, buttocks and suprapubic regions, a flat nasal bridge and prominent nares. The rest of the examination was unremarkable. Investigations showed cerebellar hypoplasia on the the cranial MRI. Chest radiograph showed cardiomegaly while the echocardiogram showed pericardial effusion. The serum transferrin isoform pattern analysis showed elevated levels of the disilo-transferrin isoform and trace levels of the asialo-transferrin isoform seen in congenital disorder of glycosylation type 1. These fractions were not seen in the normal control samples. Enzyme testing of peripheral leucocytes showed decreased level of phosphomannomutase 0.6 nmol/min/mg protein (normal 3.6-9.0) and normal level of phosphomannose isomerase 19 nmol/min/mg protein (normal 12 - 15) confirming the diagnosis of CDG type 1a. Further investigations showed impaired coagulation, hypothyroidism and persistent hypoalbuminemia. She presented with recurrent episodes of acute respiratory distress as a result of the severe pericardial effusion. In view of the impaired coagulopathy, she was transfused with fresh frozen plasma before pericardiocentesis could be performed. This procedure was repeated several times over the course of 3 months. She finally succumbed to her illness at the age of 7 months. To the best of our knowledge, this is the first confirmed case report of CDG type 1a in Malaysia. To achieve an early clinical diagnosis requires a high index of suspicion. Early investigations helped to detect most of the the major complications related to CDG type 1a. Despite her early demise, the diagnosis brought comfort to the couple as prenatal diagnosis will be possible for their future pregnancies.

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Recombinant Glucocerebrosidase uptake by human osteoblasts. *M.C Sa Miranda[2], M. Lamghari[1], C.C Barrias[1], M.A Barbosa[1].* 1) INEB-Instituto de Engenharia Biomédica, Laboratório de Biomateriais, Rua Campo Alegre 823, 4150-180 Porto, Portugal; 2) Unidade De Biologia de Lisossoma e Peroxisoma (UNILIPE), Instituto de Biologia Molecular e Celular (IBMC), Rua Campo Alegre 823, 4150-180 Porto, Portugal.

Gaucher Disease (GD) is an inherited disease, characterized by the deficient activity of the lysosomal enzyme glucocerebrosidase (GCR). This enzyme deficiency results in defective hydrolysis of glucosylceramide, which accumulates within lysosomes from macrophages, originating the so-called Gaucher cells. Considering the non-neuronopathic form of GD (type1), skeletal manifestations are among the most debilitating consequences, and nearly all affected patients have some degree of bone involvement. Enzyme replacement therapy (ERT) has been successful in treating many symptoms of type I GD but skeletal response lags behind. The lower response of bone to ERT could be the consequence of an inaccessibility of target lipid-engorged macrophages (Gaucher Cells) in bone compartments to circulating enzyme because of the loss of enzyme activity, short enzyme half life and may be enzyme internalisation by non-targeted cells within the bone compartment namely osteoblasts. To investigate whether osteoblasts cells are able to internalize the recombinant glucocerebrosidase (rGCR, cerezyme), we have used GCR inactivated MG63 human osteoblast. rGCR uptake by these cells was examined using 125I radiolabelling, western blot analysis and measurement of GCR activity. The data show that 1.78 % of administrated rGCR was internalized by depleted GCR human osteoblasts. 67.9% of internalized enzyme was detected in cell extract and 14.2% in cell membranes. Western blot analysis showed that the amount of GCR mature form was increased following the administrations of rGCR. With regards to the restored residual activity, the measurement of GCR activity showed that rGCR treated cells present 23.9% of control cells GCR activity. In conclusion, these data suggest that osteoblasts are able to internalize rGCR. Keywords: Glucocerebrosidase, Osteoblasts, Gaucher Disease, Enzyme Replacement Therapy, Lysosomal Diseases.

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Screening for Pompe Disease - A comparative study between the lymphocyte and dried blood-based assays. *Z.J. Lukacs[1], M. Deschauer[2], E. Mengel[3], R. Hartung[3], M. Beck[3], A. Keil[1].* 1) Department of Clinical Chemist, Hamburg University Medical Center, Germany; 2) University Halle-Wittenberg, Department of Neurology, Germany; 3) University Hospital Mainz, Department of Pediatrics, Germany.

Pompe Disease is an autosomal recessive disorder which results in deficiency of acid alpha-glucosidase. Early onset of the disorder is often misdiagnosed. As enzyme replacement therapy has been shown to be effective, neonatal screening for this disorder may significantly ameliorate the outcome. However, enzymatic diagnosis is hampered by the presence of several isoenzymes of which the neutrophil enzyme maltase-glucoamylase (MGA) shows a significant overlap in activity within the acidic pH range. Therefore, accurate measurement of enzymatic activity was usually done from fibroblasts and lymphocytes which are not useful for neonatal screening. Recently, Chamoles et al. described a method for the inhibition of MGA in dried blood spots (DBS). To allow an evaluation of its positive predictive value we compared results from the well-established lymphocyte assay with the DBS assay. Samples were taken from patients with confirmed Pompe disease. Lymphocytes were incubated with an methylumbelliferyl-substrate at different pH. Neglible activity was found for all patients in the acidic range while normal activity was measured at neutral pH. DBS were eluted, and aliquots were measured at pH 3.8 and pH 7.0. In addition, maltose was used as an inhibitor. More recently, acarbose, a potent mechanism-based alpha-glucosidase inhibitor, was used to inhibit non-specific enzyme activity. Direct comparison of 40 samples showed a significant increase in inhibition when acarbose was used thereby, facilitating the identification of positive samples. Furthermore, all of our Pompe patients (currently: 10) showed a significantly increased pH ratio(> 1000; normal range 200-1000) while at the same time the activity at pH 3.8 using acarbose was reduced (patients mean: 0.02 nmol/spot; normal range > 0.09 nmol/spot). In summary, we were able to demonstrate that the novel DBS assay compares well in its diagnostic value with the lymphocyte assay and therefore, may be further evaluated within a pilot study in newborn screening.

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Mitochondrial translation deficiency associated with intrauterine growth retardation and neurological deterioration. *V. SERRE, I. VALNOT, D. CHRETIEN, J. CHEBATH, A. MUNNICH, A. ROTIG.* INSERM U393, Paris, France.

Multiple respiratory chain deficiency represents a frequent and often unexplained cause of mitochondrial disorders. Among them, mitochondrial translation deficiency has been seldom described. We report here a generalized mitochondrial translation defect in a patient, born to consanguineous parents, presenting with severe intrauterine and post natal growth retardation. Permanent hyperlactatemia, hyperketonemia and normal plasma growth hormone were noted. A psychomotor retardation was apparent at two years. His neurological condition progressively worsened and he died at three years of age. Respiratory chain analysis revealed a severe combined complex I and IV deficiency in muscle and liver. Cultured skin fibroblasts and leukocytes detected a partial complex IV deficiency. Prenatal diagnosis was performed during the next pregnancy and detected a complex I and IV deficiency in the two fetus-twins and pregnancies were discontinued. The following fetus displayed normal respiratory chain activities, the pregnancy was continued and a healthy baby was born. A genome wide search allowed us to identify one chromosomal region of homozygosity on chromosome 17q25.1-qter (D17S1352-ter), possibly containing the disease-causing gene. This region (6.1 Mb) encompasses 171 genes including three genes encoding mitochondrial ribosomal proteins, namely MRPS7, MRPL38 and MRPL12. Western blot analysis was performed on total protein extracts from patient and controls fibroblasts. Immunodetection using the Total OXPHOS Complexes Detection Kit from MitoSciences showed low level of ND6 and COXII (mitochondrially encoded complex I and IV subunits) as well as core 2 and F1 (nuclearly encoded complex III and V subunits) compared to controls. The amount of SDHB subunit of complex II (the only complex entirely encoded by nuclear genes) was normal. These results are highly suggestive of a generalized mitochondrial translation defect in this pedigree. MRPS7, MRPL38 and MRPL12 are now under investigation.

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A spectrum of molecular variation of the FM03 gene, in a cohort of Italian families with trimethylaminuria: novel mutations, genotype-phenotype correlation and aplotype analysis. *T. Esposito[1], C. Lombardi[2], G. Scarano[2], S. Musumeci[3], F. Lonardo[2], A. Fiumara[4], F. Gianfrancesco[1].* 1) Institute of Food Science, CNR, Avellino, Italy; 2) Division of Medical Genetics, Benevento, Italy; 3) Institute of Population Genetics, CNR, Alghero, Italy; 4) Department of Pediatrics, University of Catania, Italy.

Fish-odor syndrome or trimethylaminuria (TMAU) is a rare inborn error of metabolism inherited in an autosomal recessive fashion that is caused by deficiency of the flavin-containing monooxygenase 3 (FMO3), required for oxidize malodorous trimethylamine (TMA) into odorless trimethylamine N-oxyde (TMAO). Individuals affected with TMAU display a strong, unpleasant, fish-like odour, due to the secretion of TMA in their breath, sweat and urine. This leads to a variety of psychosocial problems, including disruption of schooling, clinical depression, and attempted suicide. We have collected a cohort of Italian families with symptoms of TMAU containing a number of affected individuals with marked reduction of TMA oxidation and other individuals with a borderline decreased TMA oxidation. We have investigated the genetic basis of the disorder detecting a spectrum of molecular variation in the FM03 gene comprising three novel deleterious mutations and a number of previously reported mutations. The first novel mutation was a missense (I37T) that was not found in the parents, revealing that it was a de novo missense mutation. This is the first report of de novo missense mutation causative of TMAU. The second, was a single nucleotide deletion of G1182 at codon 394 that result in a frame shift and cause premature termination of the FMO3 gene after codon 404. The thirth novel mutation, (R238P) was identified in a 10-year-old boy who presented a strong body odor from the age of 10 months. Moreover, we investigated by aplotype analysis a family with mild TMAU identifying a putative causative aplotype. Finally, we failed to detect any variation in other families. Our findings support the hypothesis that TMAU is not a rare recessive disorder but rather a spectrum of phenotypes in which diet and environmental exposures can play a role in triggering symptoms.

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Galactosemia in Ashkenazi Jewish Due to Galactose-1-Phosphate Uridyl Transferase Gene deletion. *K. Muralidharan, B.W. Coffee, A. DeLorenzo, E.M. Courtney, L.N. Hjelm, C. Yu.* Dept Human Genetics, Emory Univ Sch Medicine, Decatur, GA.

Galactosemia is a common biochemical disorder caused by Galactose-1-Phosphate Uridyl Transferase (GALT) deficiency. Over 300 pathogenic variants have been reported in GALT gene. Most of these variants are rare and limited to one or few families. Some variants such as Q188R and N314D are common and have been reported in many populations. Certain variants however, appear to be common or limited to certain ethnic groups. For example S135L is the most common G allele in African Americans and L195P and Y209C appear to be limited to Caucasians. We have previously reported a large deletion of GALT gene detectable by Southern analysis in Ashkenazi Jewish galactosemia patients. This 5 kb deletion appears to involves the entire GALT gene and results in absence of GALT mRNA and protein. Lymphoblasts from patients homozygous for this deletion showed evidence of alternate pathways for galactose metabolism. Carriers for this deletion appear as homozygotes for the genotype of the other allele present in the patient. This can result in apparent discord between GALT biochemical phenotype and molecular genotype in patients apart from inconsistencies in familial studies. Molecular characterization of the rearrangement causing the deletion is reported here. A series of PCR reactions targeting the GALT gene and the flanking regions was performed using DNA from deletion homozygotes and normal individuals. This analysis identified the regions outside the GALT deletion in the patients. Using this information, the junction fragment was amplified by PCR and sequenced. This reveals a complex rearrangement involving a two part deletion involving the entire GALT gene except a part of exon 8-intron 8 and the insertion of a novel 14 bp sequence. This finding provides simplified testing for this variant and enables further studies on the origin and frequency of this variant in Ashkenazi Jewish galactosemia patients.

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High prevalence of Fabry disease in young stroke patients. *A. Rolfs[1], T. Boettcher[1], P. Bauer[3], B. Winchester[2], M. Wittstock[1], J. Pahnke[1], R. Benecke[1].* 1) Dept Neurology, Univ Rostock, Rostock MV, Germany; 2) Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust, University College London, Great Britain; 3) Department of Medical Genetics, University of Tübingen, Germany.

Fabry disease (FD) is an X-linked recessive lysosomal storage disease resulting from deficient alpha-galactosidase (AGAL) and causes an endothelial vasculopathy followed cerebral ischemia. To determine the significance of FD in the young with stroke, the frequency of unrecognized patients with Fabry disease in a cohort with of acute stroke patients was determined. Between 2001 and 2004 from 27 different clinical departments in Germany 534 consecutive, unselected German young adults aged 18 to 55 years and suffering from acute stroke were screened with informed consent for Fabry disease. In males their plasma AGAL activities was measured following by sequencing the entire AGAL gene in case of low enzyme activity. In contrast, in females the entire AGAL gene was genetically screened for mutations even if the enzyme activity was normal. Complete medical information from 478 patients (89.5%) has been sent to the investigators. 15 out 319 males stroke patients and 4 out of 215 females revealed a mutation within the AGAL gene which corresponds to a prevalence of 4.7%; and 1.8%, resp. Mean age at onset of symptomatic cerebrovascular disease is 39.6 13.4 years in the group of the male stroke patients and 42.7 14.2 years in the female group. The higher frequency of infarctions in the vertebrobasilar area correlates with more pronounced changes in the vertebrobasilar vessels like dolichoectatic pathology (33.3% vs. 5.9%). Regarding the non-neurological features male Fabry patients with stroke demonstrate a significant higher frequency of acroparesthesia, hypohidrosis and proteinuria than non-Fabry stroke patients. FD accounts for up to 5% of young males aged 18 to 55 years with stroke and about 2% in females, respectively. Fabry disease must be considered in all cases of unexplained stroke in young patients, especially in cases with the combination of infarction in the vertebrobasilar artery system and proteinuria.

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DNA-Bionanosensor: Applicability to Neonatal Screening of Hemoglobinopathies. *U. Bhardwaj[1], V. Gau[2], E.R.B. McCabe[1,3,4,5].* 1) Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; 2) GeneFluidics, Inc., Monteray Park, CA, 91754; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; 4) Mattel Children's Hospital at UCLA, Los Angeles, CA 90095; 5) Molecular Biology Institute, UCLA, Los Angeles, CA 90095.

The primary aim of neonatal screening for the hemoglobinopathies is to identify newborns with sickle cell disease (SCD) so that penicillin prophylaxis and comprehensive care can be commenced preferably before the age of two months. In the United States, most of the neonatal screening laboratories use high performance liquid chromatography (HPLC) or isoelectic focusing as the primary method for neonatal hemoglobinopathy screening. Bionanotechnology is suitable for automated, high throughput screening because it allows for movements of small amounts of fluids and therefore requires a minimal sample volume. For this purpose, we evaluated a novel bionanotechnology-based platform to identify the single base-pair mismatch that causes sickle cell disease. An amperometric system utilizing hybridization to detect PCR amplified products from wild type (AA), sickle cell disease (SS) and sickle cell carrier (AS) samples. The amperometric signal from samples with sickle cell disease and heterozygous carriers was reproducibly distinguished from wild type samples. A minimum level of baseline noise was achieved with the system. We conclude that bionanotechnology represents a sensitive method to identify patients with sickle cell disease as well as carriers using PCR amplified DNA from newborn screening dried blood specimens. We speculate that with automation, this amperometric platform provides the opportunity for high-volume, cost-effective universal screening of hemoglobinopathies. We are working to improve the sensitivity of this platform to accommodate unamplified genomic DNA from newborn screening specimens.

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High-throughput and comprehensive CHIP-based resequencing of the mitochondrial DNA in patients with OXPHOS disease. *H. Smeets[1,2], M. Gerards[1], A. Hendrickx[2], R. Van Eijsden[1], I. De Coo[3], P. Lindsey[1].* 1) Genetics & Cell Biology/GROW, Maastricht University, Maastricht, Netherlands; 2) Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands; 3) Neurology, Erasmus University Medical Center Rotterdam, Netherlands.

Mitochondrial disorders are often fatal multisystem disorders, associated with abnormalities of oxidative phosphorylation (OXPHOS). Because of its dual genetic control, defects in OXPHOS can be due to mutations in either the mitochondrial (mtDNA) or nuclear DNA. Although OXPHOS disorders have common characteristics as a group, there is considerable clinical variability among patients, even in those having the same genetic defect. Also, clinically indiscernible conditions can be caused by different mutations in a number of genes and it is often not possible to directly identify the genetic defect involved. Therefore, we applied a new CHIP-based platform suitable for rapid resequencing of the mtDNA. Up to now 30 chips have been processed in different experiments. CHIPs were analysed using both the Affymetrix GDAS software and the statistic analysis software R. We found a complete match with classical resequencing in 3 samples. Two samples were mixed to generate artificially heteroplasmy at 11 positions in the mtDNA sequence. Ten were detectable as such and one gave a no-call. Analysis of mixed amounts of different mtDNAs indicated that it should be possible to quantify a mutation load of 10% (n=3). Although the platform is not optimal for the detection of small deletions or insertions, we were able to identify two samples with a single nucleotide insertion as a heterozygote call. Screening of 20 patients suspected for mitochondrial disease revealed 171 different mutations: 90 SNPs, 5 known pathogenic mutations and 71 unknown variants (5 of which were heteroplasmic). Our conclusion is that the resequencing CHIPs are a very promising tool for rapid and comprehensive mtDNA-screening. Especially for genomes like the mtDNA, where it is possible to generate the template with a single PCR, and in which the vast majority of the point mutations are nucleotide substitutions, it will become the method of choice.

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Functional characterization of mutant ATP7B identified in Korean patients with Wilson disease. *S. Park[1], G. Kim[1,2], J. Lee[2], H. Yoo[1,2].* 1) Genome Research Ctr, Asan Medical Ctr, Seoul, Korea; 2) Medical Genetics Clinic & Laboratory, Asan Medical Ctr, Seoul, Korea.

Wilson disease (WND), an autosomal recessive disorder of copper transport, is characterized by excessive accumulation of intracellular copper in the liver and extrahepatic tissues. The mutation screening of ATP7B was performed in Korean patients with WND and ten novel mutations were identified. In order to evaluate the functional defects of ATP7B protein caused by novel mutations, we used the yeast complementation assay system and confocal microscopic evaluation for analyzing the localization of mutants after transient expression in mammalian cell system. Five novel mutations, p.C656X, p.G891D, p.V1106I, p.T1029I, and p.T1031A were constructed into the yeast expression vector, pSY114 and the mammalian expression vector, pEGFPC1. Three novel mutant constructs, p.C656X, T.1029I, and p.T1031A, were unable to rescue the ccc2 mutant yeast due to the reduced affinity of iron uptake in the yeast complementation system, indicating their defective ATP7B protein functions. The novel mutation found in a patient with less severe symptom, p.G891D construct partially restored ccc2 mutant yeast. A p.V1106I construct completely restored as wild type, which suggested p.V1106I was not a mutation but a polymorphism. In the trafficking analysis of the mutant ATP7B with confocal microscope, mutant p.C656X was scattered throughout transfected COS7 cells. A mutant p.G891D was partially localized to the site of the trans-Golgi network. Two mutants, p.T1029I and p.T1031A were predominantly mislocalized to perinuclear regions. A mutant p.V1106I was normally targeted to the trans-Golgi network, which was concordant with the result in the yeast complementation system. In conclusion, yeast complementation assay and confocal microscopic evaluation are useful tools for functional study on mutant ATP7B protein.

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Mutational Analysis of GTP cyclohydrolase I gene in the Dopa responsive dystonia. *X. Liu, J. Ye, Y. Zhang.* Dept Pediatrics, Xinhua Hosp, Shanghai, China.

Objective Dopa responsive dystonia (DRD) is a childhood onset dystonia which responds to levodopa. Mutations in the GTP cyclohydrolase I (GCH 1) gene were recently identified in number of patients with DRD. The aim of the present study was to determine the correlations between clinical expression and the mutations in the GCH-1 gene. Methods The 6 exons and exon-intron junctions of GCH-1 gene were sequenced in 9 DRD patients from 7 no kinship family by using PCR and DNA sequence analysis. The age at onset ranged from 6 years to 14 years old. Five patients were male and 4 patients were female. Results Eight of nine patients presented with dystonia and parkinsonian, which responed to levodopa. IVS5+3insT mutation was identified in 3 patients (2 female , 1 male) from one family, originated from their mother. One (male, 5 years old) of them was asymptomatic. IVS4+121(c-t) was identified in one patient. The exons and exon-intron junctions of GCH-1 gene mutations were not identified in the rest of patients. Conclusion The mutations in GCH 1 gene were only identified in 2 of 7 DRD patient's families.

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DHPLC mutation analysis of Phenylketonuria in large multi-origin population. *Y. Anikster[1], A. Elimelech[2], T. YardenI[2], S. Korem[2], G. Schwartz[1], H. Gore[2], O. Vais[2], D. Bercovich[1,3].* 1) Metabolic Disease Unit, Safra Children's Hosp, Tel-Hashomer Rama, Israel; 2) Migal - Galilee Bio-Technology Center Kiryat-Shmona,Israel; 3) Tel Hai Academic College, Israel.

The object of this research was to define the epidemiology and mutations of PAH gene (and their prevalence) that cause phenylketonuria (PKU) in different ethnic groups among the Israeli population. At the PKU center in the Metabolic Disease Unit, at Safra Children's Hospital, Sheba Medical Center, there are 450 PKU patients in treatment and follow-up, which come from 200 different families with known ethnic origin. DNA samples were collected from 174 families and mutations screening were performed using the new DHPLC technique and sequencing the DNA alterations for characterization of the mutations. Until now, all the prenatal diagnosis for the PAH gene was done by RFLP and not by specific mutation. The last study on this subject had been preformed in the late 1980's and early 1990's (up to 1992) and then a common mutation was found among Jews from Yemen, which is a deletion of part of the gene around exon-3. Since then many new patients have been added, some of them of ethnic groups whose mutations have never been characterized. In screening the 13 coding Exons of the PAH gene, we found 4 new mutations and characterize the four most common mutations (IVS10 -11G/A /N -13.59%, A300S /N -12.06%, A403V /N -9.7%, R408W /N -7.76%). About 10% of the patients were homozygous for missence mutations and 40% of the patients were found to be compound heterozygous, some with more then two mutations alleles as results from intermarried and match making. In this study we report the successful use of DHPLC to analyze rapidly the complete coding sequence of the PAH gene in a total of 174 patients from different origin. This study enables us to estimate the prevalence of the disease and the design of prenatal diagnostic tests for this disease targeted specifically to different population groups.

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Molecular analysis in MPS IIID: report of two novel mutations. *C. Beesley[1], D. Concolino[2], A. Piccirillo[2], M.T. Moricca[2], B. Winchester[1], P. Strisciuglio[2].* 1) Biochemistry,, Institute of Child Health, University College London, London; 2) Department of Pediatrics, University "Magna Graecia" of Catanzaro, Catanzaro, Italy.

The Mucopolysaccharidoses (MPS) are a family of lysosomal storage diseases caused by deficiencies of enzymes required for degradation of glycosaminoglycans (GAGs). The MPS III subfamily comprises four different groups, from MPS III A to MPS III D. The latter is the rarest of the four and is caused by a deficiency of N-acetylglucosamine-6-sulfatase (GNS), which is required for the degradation of heparan sulphate. The main clinical aspects are mental retardation with relatively mild somatic involvement and mild bone dysostosis. To date, only two mutations (R355X,1169delA) have been identified, both predicting premature termination of the protein. We present the clinical, biochemical, and molecular data of two families with MPS III D: the first family included two brothers, born from unrelated parents, with mental retardation, aggressive behaviour, macrocephaly, mild coarse face, corneal opacity and a mild degree of dysostosis multiplex. In the second family there is only one patient, with the same phenotype. The diagnosis of MPS III D, in both the families, was confirmed by increased excretion of heparan sulphate in urine and deficiency of GNS activity in skin fibroblasts. Two novel mutations were identified, one in each family. In the first family, molecular analysis of the GNS gene showed that both patients were homozygous for a large intragenic deletion of 8723bp. The deletion includes 3840bp of intron 1, the whole of exon 2, intron 2 and 46bp of exon 3. Both parents are heterozygous for the deletion. In the second family, the affected patient is homozygous for a nonsense mutation in exon 7 (CAA>TAA:Gln>Stop; Q272X). Both parents are heterozygous for the nonsense mutation. Our data showing molecular heterogeneity in our two families with the same phenotype confirms the genetic heterogeneity of MPS III D, as previously described for the other Sanfilippo subtypes.

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Complex Glycerol Kinase Deficiency (cGKD): Inversion-Indel Mutation Described By Breakpoint Junction Sequencing. *Y.-H. Zhang[1], B.-L. Huang[1], U. Bhardwaj[1], E.R.B. McCabe[1,2,3,4].* 1) Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA, 90095; 2) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA, 90095; 3) Mattel Children's Hospital at UCLA, Los Angeles, CA, 90095; 4) Molecular Biology Institute, UCLA, Los Angeles, CA 90095.

Complex glycerol kinase deficiency (cGKD), an Xp22-p21 contiguous gene syndrome, involves the GK locus together with the adrenal hypoplasia congenital (AHC) and/or Duchenne muscular dystrophy (DMD) loci. Clinical features of an individual patient with cGKD depend on the loci involved. Although many cGKD patients with deletions in this region have been previously reported and gross alterations in their Xp22-p21 regions have been described, little is known about the genomic mechanisms responsible for these intergenic chromosomal rearrangements. In a previous study of cGKD, we identified eight patients with large genomic deletions in Xp22-p21 and characterized their mutations in detail. The purpose of our current investigation was to sequence the breakpoint junctions of DNA from a patient with cGKD (GKD and AHC phenotype) and a complex chromosomal rearrangement in order to gain insight into potential mechanisms. We used PCR-based genomic walking to identify the locations of the DNA breakpoints. Breakpoint junctions were sequenced (ABI 3730), and analyses performed (NCBI and UCSC browsers). The complex mutational event in this patient involved insertion of 181 bp and deletion of 693.5 kb, associated with inversion of DNA flanked by the indel. The deletion involved complete loss of the *NR0B1* and *GK* genes, consistent with the patients phenotype. The precise mechanism of this mutation remains obscure. However, we speculate that it may involve complex loop formation by interaction of complementary sequences present in the regions of the mutation breakpoints. This mechanism would lead to an inversion associated with an indel.

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Elevated Homocysteine and micronuclei as a risk factor for recurrent abortions. *G. Vinukonda[1], R.R Akella[2], K.R prabhakara[2].* 1) Dept Pathology, New York Medical Col, Valhalla, NY; 2) Center for DNA finger printing and Diagnostics,Hyderabad, India.

Human reproduction is a remarkably critical process. A number of possible etiologies have been proposed to explain the occurrence of recurrent pregnancy loss in-patients. Current medical literature suggests that causes are identifiable in only 50% of patients. But still 50 percent of women with recurrent miscarriages remain unexplained through the routine investigations. Recently much attention has been focused on the potential role of hyper-homocystenemia in the etiology of neural tube defect, early and late complications of pregnancy loss. Case-control studies have shown mildly elevated levels of homocysteine to be present in high proportion of women with history of recurrent miscarriages suggesting it is an independent risk factor for recurrent fetal loss. The enzymatic defects involved in homocysteine metabolism and their genotypic associations with recurrent early pregnancy loss are not well studied. In the present study we investigated 68 couples with un-explained recurrent abortions and 40 controls to evaluate the potential role of homocysteine as a risk factor for recurrent pregnancy loss. The study estimates the total homocysteine levels in the plasma using HPLC with florescence detection. Micronuclei frequencies were assessed as marker for chromosomal/DNA damage in cytokinesis-blocked lymphocyte cultures. Further prevalence of MTHFR gene mutation and its association to elevated homocysteine in these case and controls were studied. Of the total group tested in the present study showed significant levels of elevated homocysteine between controls and RA. Of 68 RA females tested for MTHFR genotype, of which 1.4% was TT, 30.8% were CT and 67.6% were wild genotype and there is no record of TT genotype and 20.0% were CT and 80.0% were wild genotype recorded in controls. The genotypic differences between two groups were not statistically significant but it is observed there is increased t-allele frequency in cases than controls. An increased level of micronuclei frequencies was observed in the study group than controls and the differences were statistically significant. In summary the plasma homocysteine levels and micronuclei data supports the potential role in the early pregnancy loss in recurrent abortion cases. Further extension of our observation on the reasons/causative factors for elevated homocysteine, the MTHFR gene mutation alone is not a responsible factor in this group of recurrent abortion cases.

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MPS I Registry: Significant disease morbidity is observed in patients with the attenuated form of Mucopolysaccharidosis Type I (MPS I) or Scheie syndrome. *J. Thomas[1], G. Pastores[2].* 1) Dept Pediatrics, Univ Colorado School of Medicine, Castle Rock, CO; 2) NYU School of Medicine, New York, NY.

MPS I is a rare, inherited lysosomal storage disorder that is chronic, progressive, multisystemic, and displays extreme heterogeneity in its clinical presentation and disease progression. Despite the absence of clear clinical definitions, MPS I patients are classified into 3 syndromes: severe, early-onset Hurler (H), intermediate Hurler-Scheie (HS), and attenuated Scheie (S). As of February 2005, the MPS I Registry accrued observational clinical assessment and outcomes data from 237 MPS I patients, of whom 44% were classified as H, 25% HS, and 14% S; 16% were not classified. The median current age for H, HS, and S patients was 5.4 (1, 23.5), 12.3 (1.8, 48.8), and 23.8 (5.6, 64.5) years, respectively. Symptom onset was reported at a median age of 0.4 (0, 6.1), 2.7 (0, 8.2), and 6.6 (0.2, 33.8) years and MPS I diagnosis was established at a median age of 0.8 (0, 6.8), 3.8 (0, 36.1), and 9.9 (1.9, 54.1) years in H, HS, and S patients, respectively. The delay between symptom onset and disease diagnosis was longest and most variable in S patients, ranging from 0 to 39.5 years. Data were collected on 23 symptoms characteristic of MPS I. While the pattern of symptoms reported by S patients was distinct from H and HS patients, several symptoms were reported at similar rates among all patients, including cardiomyopathy, hepatomegaly, hernia, corneal clouding, dysostosis multiplex, scoliosis, and sleep disturbances. No S patients reported cognitive impairment, and the rates of pneumonia, kyphosis, and hearing aid use were much lower in S compared to H patients. However, cardiac valve abnormalities, joint contractures, myelopathy, and congestive heart failure occurred at higher rates in S than H patients. Cor pulmonale was only reported in S patients. We conclude that S patients experience significant morbidity related to MPS I disease and speculate that patients exhibiting S syndrome may be under-diagnosed due to the later emergence and distinct pattern of MPS I symptoms relative to H and HS.

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Identification and functional characterization of 10 novel GAA alleles causing the juvenile-adult form of Glycogen Storage Disease type II. *B. Bembi[1], A.L.E. Montalvo[1], M. Filocamo[2], A. Dardis[1], C. Danesino[3], L. Merlini[4], G. Parenti[5], D. Donnarumma[2], G. Ciana[1], M.G. Pittis[1].* 1) Metabolic Diseases Unit, Pediatric Hospital B. Garofolo, Trieste; 2) Diagnosi Pre-Postnatale Malattie Metaboliche, G.Gaslini Institut, Genova; 3) Università di Pavia, Pavia; 4) Istituto Ortopedico Rizzoli, Bologna; 5) Università Federico II, Napoli, Italy.

Glycogen Storage Disease type II (GSDII) is an autosomal recessive disorder in which deficiency of acid alpha glucosidase (GAA) results in impaired glycogen degradation that acuumulates within lysosomes. Clinically, the infantile GSDII form is the most severe subtype characterized by progressive muscle weakness and severe cardiac involvement. In the juvenile form, onset occurs after early infancy and glycogen storage predominates in skeletal muscle, usually without cardiac involvement. The adult form is similar to the juvenile form but residual enzyme activity is higher and miopathy has a slower progression. The GAA is synthesized as an inactive 110 kDa precursor which is transported to the lysosomes where it is processed into the fully active forms of 76 and 70 kDa. More than 120 mutations in the GAA gene have been described up to date (http://www.eur.nl/FGG/CH1/pompe.) We analyzed the GAA gene in 35 unrelated patients affected of juvenile-adult GSDII. Overall, we identified 22 different alleles: 10 of them are due to 12 novel mutations. Three mutations are part of a new complex allele that replaces residues 612 to 616 (HWTGD) with RGI (p.H612_D616delinsRGI). As expected, the IVS1 (-13 T/G) correlated to late onset GSDII, was the most frequent mutation, present in heterozygosis in 30/35 of the patients studied. The mutation GAA profile was characterized by mutations spread all over the sequence, including point mutations, both small and large deletions, small insertions and splicing aberrations. Functional characterization of the novel missense mutations p.A445P, p.D489N and p.H612Q was performed by enzyme assay and Western blot analysis, using a human $GAA^{-/-}$ cell line. p.D489N and p.H612Q mutants remained as the 110kD precursor expressing no enzyme activity, while the p.A445P is not even expressed or rapidly degraded.

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Lysosomal disorders in Brazil: results in 26,900 high-risk patients submitted to a screening protocol. *J.C. Coelho, M.G. Burin, K. Michelin, M. Viapiana, R. Guidobono, M. Tsao, J. Mari, T. Barcelos, A. Wajner, R.F. Pires, B. Cardoso, K. Lazzaroni, J. Huve, F.T.S. Souza, M.L. Pereira, S. Leistner-Segal, U. Matte, R. Giugliani.* Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil.

A reference centre for lysosomal storage disorders (LSD) was set up in 1982 in Porto Alegre, South Brazil. Initially the centre provided diagnosis for mucopolysaccharidoses (MPS), and progressively incorporated techniques for the identification of sphyngolipidoses, glycoproteinoses, mucolipidoses, lysosomal transport defects and Pompe disease. Methods for the identification of neuronal lipofuscinoses are presently being standardised. Enzyme diagnosis is provided for most of these diseases and identification of common mutations is also performed in selected cases. Besides patient identification, the centre provides prenatal diagnosis for most LSD and carrier detection for many diseases. Several diagnostic protocols were set up. Until now the biochemical investigation was performed in 26,900 patients and an inborn error of metabolism (IEM) was detected in 2,559 cases (9.5%). The group of IEM with higher incidence in our sample was LSD (63%), followed by aminoacidophathies (18.9%). The LSD most frequently diagnosed were Gaucher disease (471 cases), MPS II (165 cases), GM1 gangliosidosis (135 cases), MPS I (135 cases) and MPS VI (113 cases). The high relative frequency of LSD recommends the formation of regional reference centres to provide diagnosis, prevention and care for the affected families. The combined efforts of physicians (diagnostic suspicion, management), academic services (lab diagnosis, research, education), research agencies (support of projects), pharmaceutical & biotech companies (development of novel therapies, registries, clinical trials), parent associations (diffusion of information, pressure for availability of treatment) and government health agencies (providing access to treatment) is essential to reduce the burden of this important group of diseases (Support: GPPG/HCPA, PROPESQ/UFRGS, FAPERGS, CNPq, CAPES, PRONEX/MCT, NORD, TKT, BioMarin, Genzyme).

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Measurement of galactosamine and glucosamine in hydrolyzed urine as an aid to the diagnosis of Mucopolysaccharidoses. *P.A. Levy.* Department of Pediatrics, Children's Hospital at Montefiore, AlbertEinstein College of Medicine, Bronx, NY.

Intro: Mucopolysaccharidoses (MPS) are a group of lysosomal disorders characterized by excretion of glycosaminoglycans (GAGs) in urine. Patients suspected clinically of having a MPS generally have urine sent for an MPS spot test as a first step toward diagnosis. This test qualitatively detects the presence of GAGs, but may not detect all MPS patients. Other methods (dimethylene blue and carbaozole) measure the presence of abnormal quantities of glycosaminoglycans but do not identify the elevated component(s). Most labs then do TLC or electrophoresis that is able to detect the component GAGs (Dermatan sulfate, Heparin sulfate,etc). GAGs are polymers of either galactosamine,or glucosamine and glucuronic acid. If urine precipitated with CPC was subjected to acid hydrolysis, levels of galactosamine and glucosamine which are ninhydrin positive compounds would be detectable by an amino acid analyzer. For example, a patient with MPS VI would have elevated levels of dermatan sulfate (N-acetyl-galactosamine and iduronic acid). Acid hydrolysis should yield elevations of galactosamine. Methods: Samples were obtained from 5 controls, 3 MPS VI patients, also urine from previous testing was available from 2 MPS II patients as well as 1 MPS III patient. Urine was precipitated with CPC overnight. Acid hydrolysis was done with 4N HCl for 3 hours. A Beckman 6300 amino acid analyzer was used to detect the ninhydrin positive compounds. Results: Normal controls had low levels of both galactosamine and glucosamine. MPS VI patients had marked elevation of galactosamine. MPS II patients had elevations of both galactosamine and glucosamine. The one MPS III patient had expected increased levels of glucosamine, but in the range of controls. Ratios of Galactosamine to glucosamine were less than 1 for the controls and the 1 MPS III patient. MPS II patients were intermediate with a ratio of 2 to 4 and the MPS VI patients had a ratio greater than 13. It may be possible to detect and diagnose many of the MPS disorders by measuring levels of glucosamine and galactosamine in urine.

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Diagnosis of Pompe Disease by Enzyme Assay in Dried Blood Spots. *H.K.W. Kallwass, C.L. Carr, J. Keutzer.* Genzyme Corp., Framingham, MA.

INTRODUCTION. The enzymatic defect in Pompe disease is a deficiency of lysosomal acid -glucosidase (GAA) which leads to lysosomal glycogen accumulation. GAA quantitation in whole blood is problematic due to several -glucosidase isoenzymes. Maltase-glucoamylase (MGA) is of particular concern due to its low pH optimum. Current diagnostic methods circumvent this problem by using MGA-free specimens such as muscle biopsies, cultured fibroblasts or lymphocytes. These methods are invasive, slow or technically demanding. Dried blood spots on filter paper (DBS) have been used to assay many enzymes for the diagnosis of lysosomal storage diseases. Advantages of DBS are noninvasive collection, excellent enzyme stability and low laboratory costs. Here we present -glucosidase assays in DBS for rapid and highly reliable Pompe disease diagnosis. **METHODS**. MGA interference was reduced with the competing substrate maltose in an assay with 4-methylumbelliferyl--glucoside (4-MUG) (Clin. Chim. Acta 2004, v347, p97). We examined 12 infantile and 58 late-onset Pompe DBS in this assay versus a control group of 120 normal adults. Acarbose is a potent -glucosidase inhibitor with remarkable selectivity for MGA vs. GAA (Clin. Chem. 2004, v50 p1785). We developed a novel fluorescent assay protocol with 4-MUG as substrate and acarbose as inhibitor. Eighty Pompe patient DBS (58 adult and 22 infantile) were compared to 150 normal controls. The acarbose protocol was further qualified with specimens contributed by several labs worldwide. **RESULTS**. All Pompe patients were correctly identified. With the novel and superior acarbose assay we detected 4 patients who were previously misdiagnosed using conventional methods. No mutations were found in the 4 individuals by DNA sequencing of the GAA gene. **CONCLUSIONS**. With enzyme replacement therapy in the final stages of clinical development it will be crucial to diagnose infantile Pompe patients rapidly to reduce the time to start of treatment. The assays presented here will make that possible anywhere in the world. The first GAA activity study on a large cohort of late-onset patients demonstrates that this robust and cost efficient Pompe test is also well suited for diagnosing the late-onset form of the disease.

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Gaucher disease: Global gene expression profiles and the progression of pro- and anti-inflammatory pathways in mouse models. *Y. Xu, L. Jia, B. Quinn, G.A. Grabowski.* Dept Pediatrics, Div Human Gen, Children's Hosp Medical Ctr, Cincinnati, OH.

Gaucher disease (GD) is caused by defective hydrolysis of glucosyl ceramide (GC), the penultimate metabolite in the glycosphingolipid degradative pathway. The resultant accumulation of GC leads to broad functional impairments in multiple organs, but the pathogenic pathway is obscure. The presence of lipid-storage macrophages (Gaucher cells) in visceral organs is a hallmark feature of GD. To understand the molecular pathogenesis of GD, developmental global gene expression was examined by microarray analyses of total mRNAs from lung, liver, and spleen of glucocerebrosidase (GCase) point-mutated V394L/V394L (4L) and D409V/null (9V/null) mice and wild-type age-matched controls. Duplicate pooled mRNAs from three mice were analyzed using the MOE430 Affymatrix chip and ~9.5 to 17.4 % of genes showed altered expression patterns. These genes represented those of macrophage activation, signal transduction, cell adhesion, extracellular matrix protein, proteinase, transcription factor, ion transport, metabolic enzymes, and membrane proteins. Macrophage pro- (17) and anti-inflammatory (14) cytokine/mediator genes were differentially altered (>1.8-fold) in the lung, liver, or spleen of GCase 9V/null or 4L mutant mice. Progressive alterations of the INF-gamma and IL-4 pathways were similar, but to different degrees, in visceral tissues from 4L and 9V/null mice. Real-time RT-PCR, RNA dot blotting, and immunohistochemistry studies verified the altered gene expression patterns. Histopathologic analyses showed correlations of the degree of tissue/GC alterations and the gene expression profiles. These analyses implicate IFN-gamma pro-inflammatory and IL-4 anti-inflammatory networks in the temporal and differential tissue progression in GD. The disease courses and the final pathophysiology may be related to the interaction and balance of the IFN-gamma and IL-4 regulated networks.

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Phenotypic heterogeneity in type 1 Gaucher disease: A comparison between Brazil and the rest of the world. *R.F. Pires[1], E. Sobreira[2], G.A. Grabowski[3].* 1) Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; 2) FCM Santa Casa de São Paulo, São Paulo, SP, Brazil; 3) FCM Santa Casa de São Paulo, São Paulo, SP, Brazil; 3) Childrens Hospital Research Foundation, Cincinnati, OH.

Gaucher disease (GD), the most common lysosomal storage disorder, results from dysfunction of glucocerebrosidase. GD type 1 is characterized by marked phenotypic variation in age of onset and degree of hepatosplenomegaly, bony disease, and lung involvement. Most data on GD comes from the USA, Europe, and Israel, where the largest proportion of affected patients are of Ashkenazi Jewish descent. However, GD type 1 is pan-ethnic and the non-Ashkenazic variants require more thorough characterization. In 2001, Brazilian physicians began participating in the International Collaborative Gaucher Group Gaucher Registry. As of October 2004, the Gaucher Registry included demographic and longitudinal data from 3045 patients with GD type 1; 196 were from Brazil. We compared selected demographic, clinical, and genetic parameters in Brazilian GD type 1 patients with those from the entire GD type 1 cohort from the rest of the world (ROW). Overall, Brazilian GD type 1 patients were younger (<18 yrs) (65% vs 52%, p = 0.0002), included more females (67% vs 53%, p=0.0001), had more baseline anemia (57% vs 30%, p <0.0001), and had more bone pain (57% vs 36%, p=0.0004) and bone crises (19% vs 8%, p = 0.003). Fewer Brazilian patients reported Ashkenazi Jewish ethnicity (0.5% vs 39%, p <0.0001). Genotype data were available for 59% of Brazilian and 72% of ROW GD type 1 patients. In Brazil, the most common genotype was N370S/L444P (50% vs 15% in ROW, p<0.0001), whereas in ROW, N370S/N370S was the most common genotype (37% vs 7% in Brazil, p<0.0001). In Brazil, 60% of patients had at least one L444P allele vs 21% in ROW (p<0.0001). These data highlight the variation in phenotypic manifestations of GD type 1 and indicate that as a group these Brazilian GD type 1 patients have more aggressive disease than ROW patients. The findings also emphasize the need to more fully delineate GD type 1 in various groups and the need for caution in making generalizations about this disease across different demographic groups.

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Fabry Disease: Characterization of -Galactosidase A Signal Peptide Mutations. *M. McCarthy, M. Yasuda, R.J. Desnick.* Department of Human Genetics, Mount Sinai School of Medicine, New York, NY.

Fabry disease is an X-linked recessive inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal exoglycohydrolase, -galactosidase A (-Gal A). The structure of the 31 amino acid signal peptide of the -Gal A glycopeptide is consistent with that of the general signal peptide motif, having one basic amino acid within the first five, a hydrophobic core of >9 residues, an -helix breaker positioned between -4 and -8, and small neutral residues at positions -1 and -3. To date, eight -Gal A signal peptide mutations have been reported in patients with Fabry disease. M1I, M1R, and M1T mutated the initial Methionine residue, while L14P, A15E, L19R, and A20P occurred in the hydrophobic domain. A31V disrupted the -1 residue, which is important for proper signal peptide cleavage. Of note, the A20P mutation was identified in a patient with the cardiac variant phenotype, while the remaining mutations occurred in classically affected males. To characterize these signal peptide mutations, mutant constructs were generated using site-directed mutagenesis and overexpressed in COS-7 cells. The mutant proteins of M1I, M1R, M1T, L14P, A15E, and L19R had negligible -Gal A activity, and were not detectable by western blotting or immunofluorescence staining. In contrast, A20P had ~3% of mean wild-type activity and localized to the lysosome. While A31V had ~1% residual activity, the mutant protein predominantly localized to the ER, presumably due to failure of signal peptide cleavage. To determine whether the mutant proteins of L14P, A15E, and L19R were degraded by the ubiquitin-proteasome system, transiently transfected COS-7 cells were treated with the potent proteasome inhibitors, lactocystin and epoxomicin. Both proteasome inhibitors failed to show enhancement of protein levels, thus suggesting that this may not be the major enzyme degradation mechanism. Additional studies, including docking studies using the SRP 54 M domain structure and mutagenesis studies, are underway to further delineate the molecular mechanism by which these signal peptide mutations cause the enzyme deficiency.

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Molecular Analysis of the GAA gene in -glucosidase deficiency (Pompe Disease). *M.T. Geraghty[1], E. McCready[1], N. Carson[1], P. Chakraborty[1], J. Clarke[2], J. Callahan[2], R. Casey[3], A. Chan[4].* 1) Dept Genetics, Children's Hosp Eastern Ont, Ottawa,Canada; 2) Hospital for Sick Children, Toronto; 3) Alberta Childrens Hospital, Calgary; 4) University of Alberta, Edmonton.

Glycogen storage disease, type II (GSD II; Pompe disease) is an autosomal recessive disease characterized by accumulation of lysosomal glycogen within various tissues. GSDII is caused by a deficiency in the acid- glucosidase enzyme encoded by the GAA gene on chromosome 17q25.3. The enzyme is most reliably measured in cultured fibroblasts. Molecular analysis of the GAA gene would simplify the diagnosis of individuals with GSDII and permit both pre-symptomatic and prenatal testing, but there is limited availability of such testing. We developed a clinical molecular test for the detection of GAA mutations based on cycle sequencing of the complete coding region. Exons 2-20 of the GAA gene are amplified in six independent PCR using intronic primers. The resulting products were purified and sequenced. Preliminary validation studies were conducted with genomic DNA from fourteen GSDII-affected individuals from four centers across Canada. To date, 25 of 28 mutations were detected (89% detection rate). Amongst the 14 patients, there were 7 compound heterozygotes and 5 apparent homozygotes. I the remaining 2 samples, analysis has been completed for approximately 85% of the GAA gene in one patient, resulting in the detection of a single mutation. We were unable to amplify exons 2-8 from the remaining patient, while the rest of the gene was homozygous for several known polymorphisms suggesting a deletion. The mutations spanned intron 1 through exon 19 and included eight novel mutations (c.2140delC; c.1100G>C (p.Trp367Ser); c.1540G>C (p.Gly514Arg); c.2662G>T (p.Glu888X);c.2269C>T (p.Gln757X);c.1799G>T (p.Arg600Leu); c.2040G>A (splice); c.1564C>A (p.Pro522His). Consistent with previous reports, a single mutation, c.-23-13T>G (IVS1-13 T>G), was found at a high frequency in the population studied (6/14 patients). The availability of clinical molecular testing of the GAA gene will assist with diagnosis in patients, and with prenatal and carrier testing for individuals at risk of Pompe disease.

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MGC4170 is the I cell disease (mucolipidosis II) gene; mutational analysis of Japanese patients. *T. Yorifuji[1], M. Kawai[1], T. Momoi[1], J. Yorifuji[1], H. Nagasaka[2], M. Takayanagi[2], T. Nakahata[1].* 1) Dept Pediatrics, Kyoto Univ Hosp, Kyoto, Japan; 2) Dept Metabolism, Chiba Children's Hospital, Chiba, Japan.

(Introduction) Leroys I-cell disease (mucolipidosis II) is characterized by progressive psychomotor deterioration accompanied by bone/joint deformities or cardiac involvements. The disease presents very early in life, and fibroblasts of the patients have diminished activity of GlcNac-1-phosphotransferase (GNPTA), which is responsible for the correct intracellular targeting of multiple lysosomal enzymes. The gene for GNPTA previously has been mapped to chromosome 4q21-q23 by other investigators. However, there have been no publications reporting the sequence of the gene or mutational analysis of the patients. In this study, to confirm the previous mapping data, we performed a genome wide linkage analyses on Japanese families with I-cell disease, followed by the mutational analysis of a candidate gene, MGC4170. (Materials and Methods) Microsatellite markers covering the whole genome at approximately 10 cM intervals were typed on three Japanese families with multiple patients with I-cell disease. Multipoint linkage analysis was performed by using the GeneHunter 2 program under an assumption of autosomal recessive inheritance. Sequencing analysis of the candidate gene was performed by exon-by-exon amplification and direct sequencing of the amplification products. (Results and Discussion) Unlike the previous reports, the results of the linkage analysis showed that the disease maps to chromosome 12q. We have identified a candidate gene, MGC4170, within that chromosomal region of interest and performed a sequencing analysis of the gene. The results revealed multiple nonsense/frameshift mutations among affected individuals. This is the first report of mutation detection for this disorder. We conclude that the MGC4170 gene is responsible for I-cell disease. Currently we are accumulating data of the mutational analysis on additional patients. We are also in the process of confirming the results by the functional analysis.

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Fabry Disease: Natural History and Quality of Life Assessment. *D. Doheny, S. Farivar, M. Cahr, M. Banikazemi, R.J. Desnick.* Dept Human Genetics, Mount Sinai Medical Ctr, New York, NY.

Fabry disease, -galactosidase A (-Gal A) deficiency, is an X-linked lysosomal storage disease. Classically affected males (<1% -Gal A activity) present in childhood with acroparesthesias, hypohidrosis, angiokeratomas, corneal/lenticular opacities, and abdominal pain. With advancing age, renal failure and cerebrovascular and cardiovascular complications lead to early demise. Manifestations in heterozygotes (hets) are variable due to Lyonization. To assess the natural history and patient-perceived quality of life (QoL) a disease-specific questionnaire, including demographics, medical history, a Pain Questionnaire, the Fabry Disease Health Assessment Questionnaire, and the SF-12 Health Survey, was sent to 363 affected males and 380 DNA-confirmed hets. Questionnaires were received from 135 (37%) affected males (9-70 yr, mean 41.0) and 195 (51%) hets (6-78 yr, mean 41.3). Mean age at diagnosis was 25.0 yr for males and 30.3 for hets. Symptoms reported more frequently by males than hets included acroparesthesias (97 v 77%) with mean age of onset of 10.3 v 16.3 yr, hypohidrosis (97.5 v 42%), temperature intolerance (99 v 80%), abdominal pain (88 v 53%), and fatigue (66 v 43%). Males had greater activity interference than hets with acroparesthesias (80 v 26%), hypohidrosis (46 v 17%), temperature intolerance (44 v 27%), and fatigue (79 v 55%), but interference from abdominal pain was similar (28 v 27%). Among 84 males and 75 hets over 40 yr, males reported more TIAs/strokes (27 v 14%) and renal failure (55 v 5%) while cardiac involvement was comparable (72 v 72.6%). Overall, more males than hets rated their general health as poor (14.2 v 4.8%); however, both reported significant disease-related limitation of activities (16.7 v 17.4%), accomplishing less most of the time (43.2 v 36.1%), with a negative effect on general mood (27.7 v 27.5%). Thus, affected males and hets of similar mean age reported significant activity interference, emphasizing that Fabry disease has a major impact on QoL, and that QoL should be evaluated, especially in hets, as an indication for enzyme replacement therapy.

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The Natural History of Niemann Pick Disease Type A. *M. Wasserstein[1], R.J. Desnick[1], A. Aron[2], S. Brodie[3], M.M. McGovern[1].* 1) Human Genetics & Pediatrics, Mount Sinai Sch Medicine, New York, NY; 2) Neurology and Pediatrics, Mount Sinai Sch Medicine, New York, NY; 3) Ophthalmology, Mount Sinai Sch Medicine, New York, NY.

Type A Niemann-Pick disease (NPD-A) is a lysosomal storage disease resulting from the deficiency of acid sphingomyelinase (ASM) and the accumulation of sphingomyelin in cells of the monocyte-macrophage system. It is a severe neurodegenerative disease leading to death in infancy, although studies to delineate its natural history have not been reported. We serially evaluated ten patients with NPD-A (6 male, 4 female; age at entry: 3 - 6 mo) from 10 unrelated families at the Mount Sinai General Clinical Research Center between 1993 and 2004. All affected infants had a normal neonatal course and early development was unremarkable. The first symptom detected in all patients was organomegaly (median age of 3 months). Developmental milestone data was available for all 10 patients through direct examination during GCRC visits and from evaluations performed by referring neurologists or by early intervention programs. Development did not progress beyond 9, 12, 12 and 10 months for gross motor skills, cognitive development, expressive language, and fine motor skills, respectively and detailed data related to the timing of skill acquisition and loss will be presented. Non-neurologic disease symptoms were: 1) gastrointestinal problems including vomiting, intermittent periods of diarrhea and constipation, massive hepatomegaly and ascites; 2) recurrent respiratory infections and interstitial storage on chest radiograph; 3) irritability and sleep disturbances; 4) failure to thrive for weight beginning at median age of 9 months; and 5) retinal stigmata in all patients by 12 months of age. All patients had progressive increases in SGOT and SGPT, mild and progressive decline in hemoglobin and hemotocrit, normal white blood cell counts, markedly decreased platelets, and low HDL-cholesterol. The median time from diagnosis to death was 21 months and no patient survived past the age of 3 years. The cause of death was respiratory failure in nine patients and complications from bleeding in the tenth.

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Impairment of intracellular redox state in Gaucher fibroblasts: involvement of APE/Ref-1 and peroxiredoxin-1.

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Gaucher disease (GD) is the most frequent lysosomal storage disorder due to an autosomal recessive deficiency of acid -glucosidase that leads to glucosylceramide (GlcCer)accumulation. The mechanisms underlying pathophysiology of GD are still not fully understood. GlcCer is a central intermediate in the synthesis-degradation pathways linking ceramide with complex glycosphingolipids. An alteration in this balance might perturb the cell metabolism contributing to the pathological phenotype. Glycosphingolipids are involved is the generation of reactive oxygen species (ROS). GlcCer accumulation increases Ca^{2+} mobilization in GD. As Ca^{2+} signalling interplays with ROS production, ROS levels are likely to be increased in these experimental models. We analyzed ROS levels in cultured GD and control fibroblasts to determine if an altered redox state might contribute to GD pathogenesis. ROS levels are 2-3 folds higher in GD cells respect to controls and revert by treatment with antioxidants; reversion of GD phenotype is almost complete in the presence of a specific blocker of NADPH oxidase, suggesting a role of this complex in ROS generation in GD. Since GD cells are subjected to a chronic oxidative stress, we analyzed systems involved in the protection of the cell from oxidative damage. In particular, we investigated the expression levels of APE/Ref-1 and Prx1. APE/Ref-1, inducible by oxidative stress, is a bifunctional protein involved in the repair of damaged DNA and in the transactivation of several transcription factors, while peroxiredoxin-1 is a ROS scavenger. Cytoplasmatic APE/Ref-1 and Prx11 levels are increased in both GD cells and control cells treated with CBE, a competitive irreversible inhibitor of acid -glucosidase. Moreover, the addition of exogenous -glucosidase (Cerezyme) reverts the Ape/Ref-1 induction in GD cells. Our data indicate that oxidative stress plays a role in the pathogenesis of GD, providing useful knowledge for the development of new therapeutic approaches.

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The cognitive phenotype of Niemann-Pick type C (NPC): neuropsychological characteristics of patients at baseline in a clinical trial with oral miglustat. *V.J. Hinton[1], D. Vecchio[1], H. Prady[2], E. Wraith[2], M.C. Patterson[1].* 1) Columbia University, New York, NY, USA; 2) Royal Manchester Children's Hospital, Manchester, UK.

Background: NPC is an autosomal recessive disorder whose phenotype is progressive neurodegenerative disease with highly variable expressivity. Isolated reports exist on the cognitive deficits in NPC, but no systematic series. We report neuropsychological findings in a cohort of NPC patients at entry into a therapeutic trial of miglustat. **Methods**: Fourteen participants with NPC (12-43 years) were evaluated to define their cognitive/behavioral phenotypes. Measures of language function (receptive vocabulary, naming and verbal fluency), visuospatial abilities (matrices and drawing), memory, and executive function were included. Scores were standardized relative to age-matched norms and classified as "severely impaired" (SI) when more than 2 SD from mean, and "mildly impaired" (MI) when more than 1 SD from mean. **Results**: At study entry, all participants exhibited significant cognitive deficits. Receptive vocabulary skills were relatively preserved (4/14 scored in the MI range; 10/14 were within normal limits). In contrast, expressive language was impaired (Naming: 5/14 SI, 7/14 MI; Verbal Fluency: 14/14 SI). Nonverbal skills were also defective (Matrices: 5/14 SI, 8/14 MI; Construction: 14/14 SI), as were executive skills (Trails: 14/14 SI; Digit Span: 12/14 SI, 2/14 MI). Severely impaired memory was observed in 12/14 participants, while the remaining two had intact memory. **Conclusions**: 12/14 NPC patients who met criteria for dementia at entry into a therapeutic trial of miglustat had significant memory deficits and impairments in expressive language, visuospatial skills and executive functions. All subjects had generalized cognitive impairment and exhibited lack of insight into the degree of deficit, posing significant challenges in daily activities.

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Serum levels of Osteoprotegerin and Osteoprotegrin polymorphisms in Gaucher disease. *G. Altarescu[1], I. Mazel[2], E. Lebel[3], M. Itzchaki[3], B. Rudensky[4], A. Zimran[2], D. Elstein[2].* 1) Genetic Dept, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem Israel; 3) Department of Orthopedics, Shaare Zedek Medical Ctr, Jerusalem Israel; 4) Laboratory for Microbiology and Immunology, Shaare Zedek Medical Ctr, Jerusalem Israel.

Bone involvement in Gaucher disease causes considerable disability and reduced quality of life; indeed, skeletal involvement, particularly loss of function and unremitting pain, are important indications for enzyme replacement therapy. Osteoprotegerin (OPG) decreases osteoclast activity. The purpose of this study was to ascertain whether OPG may serve as a marker of incipient bone involvement by comparing OPG serum levels relative to Gaucher severity score index (SSI) and bone mineral density (BMD), and to correlate various bone and Gaucher disease markers to OPG polymorphisms. Three common polymorphisms have been characterized in the OPG gene: OPG1-2A163G, 3-4OPGT129C, and 5-6C1217T. There is a predilection for the non-neuronopathic form (type I) Gaucher disease among Ashkenazi Jews. Of a total of 554 patients in a large referral clinic, 173 Ashkenazi Jewish patients with type I Gaucher disease were enrolled in the study and a control group of 32 healthy Ashkenazi Jews was also recruited. Serum OPG levels were detected by specific ELISA analysis. BMD was obtained by Dual X-ray Absorptiometry (DEXA). OPG polymorphisms were determined in 63 patients chosen at random. There was a statistically significant decrease in OPG values in patients as a function of age, and between OPG5-6 polymorphism and SSI. A statistically significant difference was also found between allele distributions of each OPG polymorphism compared to the general population and to healthy Ashkenazi Jews. However, there was no correlation between OPG levels and BMD in patients, neither at the lumbar spine nor femoral neck, nor with genotype or other markers of severity of Gaucher disease, implying OPG may not be the mechanism which could mitigate against bone loss due to Gaucher disease and/or that bone involvement in Gaucher disease is osteoclast but non-OPG mediated.

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Amino Acids in Arginase Double Knockout Mice Demonstrate Ornithine Auxotrophy. *J.L. Deignan[1], P.K. Yoo[1], J.C. Livesay[1], S.I. Goodman[2], W.E. O'Brien[3], R.K. Iyer[1], S.D. Cederbaum[1], W.W. Grody[1].* 1) Departments of Pathology and Laboratory Medicine, Psychiatry, Pediatrics and Human Genetics and The Mental Retardation Research Center, David Geffen School of Medicine at the University of California, Los Angeles, CA; 2) Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Knockout mouse models have been created to study the consequences of arginases AI and AII deficiencies, both individually and combined. The AI-/- knockout animals die by 14 days of age from hyperammonemia, while the AII-/- knockout has no obvious phenotype. The double knockout (AI-/-/AII-/-) has the phenotype of the AI-deficient mice, the absence of the AII gene product apparently not exacerbating the clinical abnormality. Plasma amino acid measurements in the double knockout have shown arginine levels increased 35-fold and levels of ornithine decreased 5-fold as compared to wildtype controls. Liver ornithine levels were reduced to 3% of normal, with arginine very highly elevated. Amino acid levels in other tissues were not changed remarkably. Amino acid analysis in the double knockouts revealed that argininosuccinate levels in liver were increased, indicating that AII may play a role in mitigating AI deficiency. Thus, we have shown that fatal hyperammonemia in the mouse AI knockout model is almost certainly due to ornithine deficiency, the amino acid needed to drive the urea cycle. The high level of expression of ornithine aminotransferase (OAT) is rapidly diminishing at this time, indicating that this enzyme is critical to the maintenance of ornithine homeostasis, at least at this early stage of mouse development. Although most human AI-deficient patients have no symptomatic hyperammonemia at birth, it is possible that clinically significant, but opaque, ornithine deficiency is present in these patients' livers as well.

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Congenital Hyperinsulinism: Correlation between pancreatic [18F]Fluoro-L-DOPA PET and immunohistochemistry. *P. de Lonlay[1], M. Ribeiro[2], A. Simon-Carre[1], N. Boddaert[1], I. Giurgea[1], C. Bellanné-Chantelot[4], J.J. Robert[1], J. Rahier[3], D. Seidenwurm[5], C. Nihoul-Fékété[1], F. Brunelle[1], F. Jaubert[1].* 1) Hosp Necker-Enfants Malades, Paris, France; 2) ERM 0205 INSERM-CEA, Service Hospitalier Frédéric Joliot, Orsay, France; 3) Department of Pathology, University of Louvain, B-1200 Brussels, Belgium; 4) Department of Biology, Hôpital Saint-Antoine, Paris, France; 5) Radiological Associates of Sacramento, Sutter Medical Center, Sacramento, California, USA.

Congenital hyperinsulinism (HI) is characterized by profound hypoglycaemia related to inappropriate insulin secretion. Focal and diffuse forms of hyperinsulinism share a similar clinical presentation, but their treatment is dramatically different. Until recently, preoperative differential diagnosis was based on pancreatic venous sampling (PVS), an invasive and technically demanding technique. Positron emission tomography (PET) after injection of [18F]Fluoro-L-DOPA has been evaluated for the preoperative differentiation between focal and diffuse HI, by imaging uptake of radiotracer and the conversion of [18F]Fluoro-L-DOPA into dopamine by dopa decarboxylase (AADC). Here, we show that the focal localization of dopamine in 4 focal HI and the diffuse localization in 3 diffuse HI were fully confirmed by immunohistochemical detection of AADC in all pancreatic surgical specimens, with diffuse staining of Langerhans islets in the whole pancreas in all diffuse cases, compared to dense focal staining in all focal cases. Staining of Langerhans islets outside the focal lesion were diffusely but weakly positive. We also correlated the localization of AADC and proinsulin in normal human pancreas and in tissue from both diffuse and focal HI. One child suspected of having diffuse HI by PVS was treated with an inhibitor of AADC (carbidopa). The diffuse uptake found before treatment disappeared completely after carbidopa, demonstrating in vivo that pancreatic cells can take up amine precursors and contain AADC responsible for the conversion of [18F]Fluoro-L-DOPA into dopamine. We validate PET with [18F]Fluoro-L-DOPA as a reliable test for the distinction of diffuse and focal forms of HI.

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Keratan sulfate accumulation and secondary elastin binding protein defect in a GM1-gangliosidosis patient with juvenile form. *A. Caciotti[1], M.A. Donati[1], A. d'Azzo[2], E. Zammarchi[1], A. Morrone[1].* 1) Department of Pediatrics, Azienda Ospedaliero-Universitaria Meyer, Florence, Italy; 2) Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN, USA.

GM1 gangliosidosis is a neurodegenerative lysosomal storage disorder caused by beta galactosidase deficiency. Beta-galactosidase gene gives rise to the lysosomal beta-galactosidase enzyme and to the elastin binding protein, whose primary deficiency is linked to impaired elastogenesis. In addition, galactosugar-bearing moieties have been demonstrated to alter elastin binding protein function and in turn to cause impaired elastogenesis. We mainly focused in this study on elastogenesis in fibroblasts from patients with the juvenile form of the disease showing a large urinary keratan sulfate excretion together with connective-tissue abnormalities. In this patient both genetic lesions identified (R201H/C230Y) affect only the lysosomal beta galactosidase. Elastic fiber assembly was evaluated by immunofluorescence studies on normal and GM1 gangliosidosis patients' fibroblasts. In the juvenile patient, a mild elastin binding protein reduction and a decreased impaired elastic fiber assembly were detected. The addition of keratan sulfate to normal fibroblasts led to decreased elastin deposition, whereas keratanase restored normal elastostogenesis in the juvenile patient's fibroblasts. These data showed that keratan sulfate accumulation leads to impaired elastogenesis with secondary elastin binding protein deficiency in the fibroblasts of the GM1 gangliosidosis patient with juvenile form. Grant: Prin2004; AMMEC and MPS Italy are gratefully acknowledged.

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Life expectancy in Type 1 (non-neuronopathic) Gaucher disease. *N. Weinreb[1], P. Deegan[2], K. Kacena[3], P. Mistry[4], G.M. Pastores[5], S. vom Dahl[6].* 1) Univ Research Foundation, Hollywood, FL; 2) Addenbrooke's Hospital, Cambridge, UK; 3) Genzyme Corporation, Cambridge, MA; 4) Yale Univ School of Medicine, New Haven, CT; 5) New York Univ School of Medicine, New York, NY; 6) St. Franziskus-Hospital, Cologne, Germany.

Objective: Investigations were conducted to estimate life expectancy at birth of patients with Type 1 (non-neuronopathic) Gaucher disease (GD). **Methods**: The GD population included all patients with Type 1 GD registered in the International Collaborative Gaucher Group (ICGG) Gaucher Registry who were diagnosed after 1991. Life expectancy was calculated according to the standard life table method (Palmore and Gardner, 1996) (method 1). Life expectancy was also calculated using the assumption that risk of death prior to Gaucher diagnosis was the same as the reference population (method 2). The reference population (World Population Prospects, 2002, UN) was males and females from the U.S. population. Approximately 40% of GD patients came from the U.S. The life expectancy of the reference population was similar to that for developed nations (as defined by the UN). The distribution of males and females in GD is similar to that of the general population. **Results**: The Type 1 Gaucher population of 2,201 patients had 90 reported deaths. The average life expectancy of the Type 1 GD population was 66.6 years for method 1, and 67.3 years for method 2. The life expectancy of the reference population was 77.1 years. **Discussion and Conclusion**: The ICGG Gaucher Registry represents the single largest dataset on GD patients worldwide. The current life expectancy at birth of people with Type 1 GD is about 10 years less than the reference population, confirming that Type 1 GD is not a benign disorder. Additional analyses of life expectancy, causes of death, and effect of enzyme replacement therapy on life expectancy are underway. Reference: Palmore JA, Gardner RW. Measuring Mortality, Fertility and Natural Increase. A self-teaching guide to elementary measures. Honolulu, Hawaii: East-West Center Publisher, 1996, pages 35-61.

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Ethylmalonic encephalopathy: Delayed biochemical and physical manifestations affect newborn diagnosis. *J. Bartley[1], D. Geary-Hook[1], P. Rinaldo[2], V. Tiranti[3], W. O'Brien[4], M. Zeviani[3].* 1) Dept Ped/Div Human Gen, Univ California Irvine Med Ctr, Orange, CA; 2) Dept Medical Genetics, Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 3) Unit of Molecular Neurogenetics, Pierfranco and Luisa Mariani Center of the Study of Children's Mitochondrial Disorders, National Neurological Institute "Carlo Besta", Milan, Italy; 4) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Ethylmalonic encephalopathy (EE) is an autosomal recessive disorder caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. EE is characterized by recurrent petechiae, orthostatic acrocyanosis, chronic diarrhea, neurodevelopmental delay and regression, prominent pyramidal and extrapyramidal signs. Death is usually in the first decade. There is persistent lactic acidemia, elevated $C_{4\text{-}5}$ plasma acylcarnitines, markedly elevated urinary excretion of ethylmalonic acid (EMA) and elevated $C_{4\text{-}6}$ acylglycines. Symmetrical necrotic lesions in the deep gray matter structures are the main neuropathological features. We report two Mexican hispanic siblings with EE born in California. The boy at two years of age had features of EE. He was placed on a low protein diet, riboflavin and a formula devoid of selected amino acids. This diet decreased hospitalizations, but he deteriorated neurologically and died at nine years of age. His sister at birth did not have any clinical manifestations of EE. Her urine organic acids (UOA) contained no EMA at two days and at two months of age. Newborn screening using tandem mass spectrometry was not done in California when she was born. Developmental delay at four months of age was noted and the UOA had a large concentration of EMA. She is a compound heterozyote for two missense mutations of ETHE1 (406 A>G, T136A and 488 G>A, R163Q). ETHE1 was present on a Western blot and she was homozygous for the 625G allele (the allele not associated with elevated EMA) of the SCAD gene. Conclusion: The delayed accumulation and excretion of EMA could be an indication of potential false negative results by newborn screening. Retrospective analysis of the original newborn screening card is underway.

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Carnitine palmitoyltransferase II deficiency: molecular aspects in twenty-five unrelated families. *J.H. Ding, B.Z. Yang, D.S. Roe, L. Sweetman, C.R. Roe.* Institute of Metabolic Disease, Baylor University Medical Center, Dallas, TX.

Carnitine palmitoyltransferase II (CPT II) deficiency, an autosomal recessive disorder of fatty-acid -oxidation, has three distinct clinical forms: the adult-onset (muscular) form, milder infantile form and severe neonatal form which may result in sudden unexplained death. In this study, twenty-five unrelated patients (probands) with CPT II deficiency were investigated. In vitro probe analysis with [16-$^{2}H_{3}$] palmitic acid from the probands fibroblasts resulted in labeled acylcarnitines consistent with CPT II deficiency. CPT II activities in lymphocytes and/or fibroblasts were measured (0.02-0.12 nmol/min per mg protein) and found to be 2-13% of mean control value. To investigate the molecular basis of CPT II deficiency, all of CPT II exons from probands DNA were amplified and directed sequenced. The common mutation S113L was detected in fifteen families, including five homozygous and ten compound heterozygous. The R124X and Q413fs were detected in four CPT II families respectively. The other mutations, including novel mutation T165I and P595fs, were identified in individual patients, and increased the number of different mutations to a current total of 44. There is significant phenotypic heterogeneity in CPT II deficiency, even within the same family. Interestingly, a probands father also notes myopathy caused by heterozygosity for single mutation Q413fs. This report demonstrated the genetic heterogeneity that may underlie the clinical variation in CPT II patients.

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Heme Biosynthesis and Porphyria: Demonstration of a Cytosolic Complex Involving Uroporphyrinogen Synthase and Hydroxymethylbilane Synthase. *L.F Cunha, M. Kuti, MM. Zhou, R.J. Desnick, D.F. Bishop.* Human Genetics, Mount Sinai Sch Med, New York, NY.

Uroporphyrinogen synthase (UROS) and hydroxymethylbilane synthase (HMBS) are the third and fourth enzymes in the heme biosynthetic pathway and both are required to synthesize uroporphyrinogen III (UROgen III) from porphobilinogen (PBG). HMBS deaminates and polymerizes four molecules of PBG to form the linear tetrapyrrole hydroxymethylbilane (HMB), which UROS then cyclizes with inversion of ring D to form UROgen III. HMB has a short half-life of ~4 min, and in the absence of UROS, it non-enzymatically cyclizes to the non-physiologic and toxic UROgen I isomer. UROS gene mutations that cause congenital erythropoietic porphyria result in decreased UROS activity and the accumulation of UROgen I. It has been suggested that UROS and HMBS interact in the cytoplasm to form UROgen III. Previously, we observed a marked kinetic enhancement of UROS in the presence of HMBS, suggesting an enzyme-enzyme interaction. However, we were unable to demonstrate a complex by yeast two-hybrid and co-immunoprecipitation experiments, which detect interactions in the M range, but not weaker mM interactions. Using nuclear magnetic resonance (NMR) spectroscopy, we demonstrated the direct physical association between UROS and HMBS in the synthesis of UROgen III based on chemical shift perturbation as observed in the titration of [^{15}N,^{2}H]-labeled purified recombinant UROS with the purified recombinant HMBS apoenzyme (E) and its mono- (ES_1), di- (ES_2) and tri- (ES_3) substrate-enzyme intermediates. UROS residues perturbed by HMBS were localized mostly in and around the active site. Results from multiple experiments demonstrated an enzyme interaction proportional to the length of the growing pyrrole chain (ES_3 ES_2>ES_1>E) attached to HMBS. These studies indicate that HMBS directly interacts with UROS in the synthesis of HMB, and then hands off HMB, thereby avoiding its non-enzymatic cyclization to the UROgen I isomer. Thus, an efficient enzyme-enzyme interaction evolved to normally prevent the formation of the UROgen I isomer, which causes the disease pathology in congenital erythropoietic porphyria.

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Evaluation of the glycosylation status of alpha-dystroglycan in Hereditary Inclusion Body Myopathy (HIBM).

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HIBM is an adult onset autosomal recessive neuromuscular disorder characterized by slowly progressive myopathic weakness and atrophy. HIBM is caused by mutations in GNE, coding for UDP-GlcNAc 2-epimerase/ManNAc kinase, the bifunctional, rate-limiting enzyme in sialic acid synthesis. Loss of activity due to GNE mutations impairs sialic acid production, which may interfere with proper sialylation of glycoconjugates. We investigated how this would lead to muscle pathology. First, we showed normal iso-electric focusing (IEF) patterns of transferrin in HIBM patients serum, suggesting normal N-linked glycosylation. Next, we performed immunohistochemistry on HIBM muscle employing antibodies against components of the dystrophin-glycoprotein complex. -dystroglycan and laminin- 2 showed normal patterns, but antibodies against O-linked glycan epitopes of alpha-dystroglycan (-DG) showed reduced staining. -DG contains both O-GalNAc and O-mannose linked glycans; the latter are rare in mammals. Next,we showed normal IEF patterns of apolipoprotein C-III, which contains only O-GalNAc linked glycans, suggesting that O-GalNAc glycosylation is unaffected in HIBM. We conclude that incomplete O-mannosylation of -DG is the underlying defect in the pathology of HIBM, as was previously shown for other muscular dystrophies. Since HIBM is an adult onset disease, and we have shown residual GNE activity in patients, the effects of sialic acid deficiency may appear gradually. When a shortage of sialic acid occurs, some glycans (e.g.,N-linked, O-GalNAc linked, glycolipids) could be preferentially sialylated over the very rare O-mannose linked glycans. O-mannosylation of -DG might only occur in skeletal muscle; other tissues are not affected in HIBM. Understanding the function and regulation of the O-linked mannose pathway is essential for developing diagnostic tests and therapies for HIBM and other muscular dystrophies with similar pathology.

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Novel *IVD* mutation in kindred with chronic intermittent isovaleric acidemia. *H. Gore, O. Vais, B. Ben-zeev, T. Shalavi, Y. Anikster.* Metabolic disease unit, Safra children's hospital, Sheba medical center, Ramat-gan, Israel.

Isovaleryl-CoA dehydrogenase (IVD) is a mitochondrial enzyme involved in the catabolism pathway of leucine. Impairment of the IVD activity causes isovaleric acidemia (IVA), a rare autosomal recessive disease. IVA can present in an acute form, which starts several days after birth, or in a chronic form. Both forms are characterized by high accumulation of isovaleric acid which is toxic to the CNS and thus can cause retardation, loss of motor skills, seizures, coma and eventually death. Other clinical features are ketoacidosis, hyperglycinemia, thrombocytopenia, leukopenia and "sweaty feet odor" during episodes. The clinical management is by restricted protein diet and administration of glycine supplementation.

We studied two siblings, aged 14 and 16, sons of first-cousin healthy parents from a Moslem Arabic origin. They both attend a regular school, with specific educational aids. Both siblings presented with vomiting episodes before age 1, but the diagnosis was made only after 4 years. The diagnosis was made by organic acid profile and high isovalerylcarnitine on both brothers (10.5 nmol/ml and 7.5 nmol/ml, normal less than 0.5).

RNA and DNA were purified from whole blood of the two siblings and their mother. The RNA was reverse-transcripted to cDNA, and the cDNA was amplified by PCR with primers to the *IVD* gene. Analysis of the *IVD* cDNAs sequences showed a homozygous missense mutation in both siblings, 774 C>G. The mother has been found heterozygote to this mutation. To confirm our finding, PCR amplification of the genomic DNA was carried out on exon 7, where the mutation should be located. A restriction enzyme assay on the PCR products revealed that both the siblings are homozygote to the mutation Cys258Trp (C258W), and their mother is heterozygote. This study brings to 17 the reported mutations in the *IVD* gene. Furthermore, it offers a quick and non-invasive pre and peri-natal diagnosis of the IVA patients and carriers, thus enables early treatment.

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Mutations of argininosuccinate synthetase gene in two citrullinemia patients with different phenotypes. *Z.Q. Qiu[1], C. Obie[2], A. Hamosh[2], T. Wang[2], D. Valle[2].* 1) Peking Union Medical College Hospital; 2) Johns Hopkins School of Medicine.

Citrullinemia is caused either by deficiency of arginonosuccinate synthetase (*ASS*) (type I) or of citrin, an aspartate/glutamate transporter in the inner mitochondrial membrane encoded by *SLC25A13* (type II). Classical type I citrullinemia presents in the neonatal period with hyperammonemia and markedly elevated plasma citrulline while type II usually presents in young adults with similar but less severe biochemical abnormalities. The advent of tandem mass spectrometry (MS/MS) has resulted in recognition of citrullinemic infants with phenotypes that vary from these classical presentations. We investigated the molecular basis of 2 such infants.Patient 1 was identified by newborn screening at age 4 d and had an $_{NH_4}$ of 200 M. He responded to standard therapy and has done well clinically despite maintaining citrulline levels up to 6000M (control 10-34M). Patient 2 was asymptomatic with mild hypercitrullinemia (200-300 M) and normal ammonia, which have persisted over the 1st year. To explain these abnormalities, we used RT-PCR to amplify the complete coding regions of *ASS* and *SLC25A13*. Direct sequencing of the RT-PCR products with confirmation in genomic DNA revealed 3 novel exonic *ASS* mutations. Patient 1 was a compound heterozygote for D124N and R272L; patient 2 was a heterozygote for a 3 bp deletion S247 (S247). We did not find these *ASS* alleles in 68 controls nor did we find a second pathological allele in patient 2. To determine the functional consequence of these mutations, we utilized an intact cell assay to measure the incorporation of ^{14}C-citrulline into protein bound ^{14}C-arginine. Cells from patient 1 showed a severe deficiency (3.6% of control) while cells from patient 2 had an intermediate value (51% of controls).We conclude that *ASS* mutations are responsible for the abnormal urea cycle function in both patients and that patient 2 is either a heterozygote for a dominant-negative *ASS* allele or a compound heterozygote for S247 and an allele we have not yet detected. Family studies to distinguish between these possibilities are in progress.

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Identification of novel mutations in patients with medium-chain acyl-CoA dehydrogenase deficiency. *B.Z. Yang, J.H. Ding, L. Sweetman, C.R. Roe.* Institute of Metabolic Disease, Baylor University Medical Center, Dallas, TX.

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common inherited defect of fatty acid -oxidation, characterized by episodes of illness in early childhood. The disorder may present after fasting with symptoms resembling Reye Syndrome, coma, hypoglycemia, hyperammonemia, fatty liver, and sudden death. The reported cases show significant phenotypic heterogeneity of MCAD deficiency, even with the same genotype. Recently, we identified eight novel MCAD mutations in our group, including the patients detected by newborn screening in our institute. The diagnoses were based on the dried blood spot acylcarnitine profile that showed highly elevated octanoyl carnitine and decenoyl carnitine, indicating MCAD deficiency. All babies with MCAD deficiency detected by newborn screening were asymptomatic prior to testing. To investigate the molecular aspect, the common mutation 985A>G had been tested, which presented in 84% of all defective alleles in our group. To screen for unknown mutations within the MCAD gene, all 12 exons and their flanking intronic sequences were amplified from probands DNA. The PCR products were purified and sequenced. Sequence analysis revealed eight novel mutations, including E43K and R349X. All novel mutations were verified by the PCR/restriction tests, but were not detected in the normal control subjects. In addition, the correlation of genotype to clinical phenotype was discussed.

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Novel Mutations Underlying Argininosuccinic Aciduria. *F. Imtiaz[1], M. Al-Sayed[2], O. Alsmadi[1], H. Khalil[1], M.S. Rashed[2,3], B.F. Meyer[1].* 1) Aragene, KFSH, Riyadh, Saudi Arabia; 2) Department of Medical Genetics, KFSH, Riyadh, Saudi Arabia; 3) Department of Genetics, KFSH, Riyadh, Saudi Arabia.

Argininosuccinic aciduria (ASA) is common in Saudi Arabia as a consequence of extensive consanguinity. It is the most common urea cycle disorder identified by the Metabolic Screening Laboratory of our Institution. Affected patients are confirmed biochemically by Tandem Mass Spectrometry, which whilst sensitive, cannot be used for carrier detection. Establishment of the range and incidence of mutations underlying ASA in this population were not previously established. We utilized Whole Genome Amplification, PCR and direct sequencing to identify the molecular lesions underlying ASA. A missense mutation (Q354X) that accounts for 50% of Saudi patients with ASA was recently reported by our laboratory. In this study we report a further four novel mutations (D115Y, G157R, R186W and G361X) found in Saudi patients with ASA. The missense and one nonsense mutation were confirmed by their absence in ~300 chromosomes from the normal population. Cross species conservation of amino acid residues was observed in some but not all instances. Where parental and other familial DNA samples were available, segregation of the mutation in an autosomal recessive pattern of inheritance was confirmed. Together the five mutations described above cover ~90% of ASA patients in Saudi Arabia. This coverage provides efficient molecular diagnosis of ASA in the Saudi population and lays the foundation for preventative measures including inductive screening in extended families, counseling, and regional pre-marital screening. In addition, identification of these mutations enables provision of pre-implantation and prenatal diagnosis as appropriate.

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Neonatal presentation of adenylosuccinate lyase deficiency. *M. Zikanova, J. Krijt, L. Dvorakova, H. Hartmannova, J. Zeman, S. Kmoch.* Inst. for Inh. Met. Disorders, Charles University, Prague 2, Czech Republic.

Adenylosuccinate lyase (ADSL) deficiency (OMIM 103050) is an inherited metabolic disease affecting predominantly central nervous system. Affected patients show first combination of hypothony and epileptic seizures and later progress with psychomotor retardation, muscular wasting and behavioural changes such as autism, aggressiveness or self-mutilation. The clinical symptoms develop gradually, usually within the first months of life. Recently we diagnosed two patients born after uneventful pregnancies at 40th and 38th week of gestation who presented with atony, acrocyanosis, bradycardia and pharmacoresistant epileptic seizures at birth. Both patients died at age of 9 and 6 weeks, respectively. Selective metabolic screening was positive for urinary SAICAR (TLC) and the ADSL deficiency was confirmed by presence of S-Ado and SAICAr in urine and cerebrospinal fluid (S-Ado/SAICAr ratios were 0,5 in both patients) and reduced ADSL activity in fibroblasts. Mutation analysis of ADSL gene showed that both patients were compound heterozygotes for Y114H/R426H and Y114H/E376D mutations, respectively. Expression studies performed in E.coli revealed that Y114H mutation leads to profound protein instability (Y114 is a residue essential for enzyme subunits assembly), while the R426H and E376D mutations substantially reduce the protein activity. The Y114H/R426H and Y114H/E376D combinations however represent the most severe genotypes observed in our serie of thirteen ADSL deficient patients. Its association with neonatal presentation and early death corroborates our previous finding that predicted residual ADSL activity, calculated as a mean of homoalelic in-vitro expressed enzyme activities, may correlates with the severity of clinical symptoms. Our work further allowed successful prenatal diagnosis in one of the families and shows that ADSL deficiency must be considered also in neonates with neurological presentation.

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Apparent B_{12} Responsive *mut^0* Methylmalonic Acidemia in Two Patients. *M.M. Martin[1], G.M. Enns[2], T.M. Cowan[3], D.S. Rosenblatt[4], S. Packman[1], R.C. Gallagher[2], K. Weisiger[1].* 1) Department of Pediatrics, University of California, San Francisco; 2) Department of Pediatrics, Stanford University; 3) Department of Pathology, Stanford University; 4) Department of Human Genetics, McGill University.

Introduction: Isolated methylmalonic acidemia (MMA) is due to deficient activity of methylmalonyl-CoA mutase and is caused by either a defect in the apoenzyme or in the synthesis of its cofactor, cobalamin (Cbl). Defects in the apoenzyme are divided into two types based on the amount of enzyme activity detected in cultured fibroblasts; *mut^0*, with undetectable activity, and *mut$^-$*, with residual activity. Responsiveness to supplemental Cbl, is generally thought not to occur in *mut^0* patients and only rarely in *mut$^-$*. We present two patients with *mut^0* MMA who both appear to be responsive to vitamin B_{12}. **Case 1** is an Asian male infant who presented on day 4 with tachypnea and lethargy. He was found to have neutropenia, acidosis and hyperammonemia (1,227 M, normal <50). Initial MMA level was 1,477 M (normal <0.3) with a normal total homocysteine. Hemodialysis (HD), hydroxocobalamin (OHCbl), and IV carnitine were started with good response. OHCbl was continued at 2 mg IM daily and MMA levels have ranged from 10 to 150 M. At 7 months of age, he has had nor further hosptializations. **Case 2** is an Asian male infant who presented on day 4 with increasing lethargy, poor feeding and hypothermia. He was found to have hyperammonemia (NH_3 795 M), metaoblic acidosis, lactic acidemia and neutropenia. Initial MMA level was 362 and total homocysteine was normal. He responded well to HD and CVVHD, nutritional support, ammonia-scavaging medications, and supplementation with carnitine, vitamin C and OHCbl (1 mg IM daily). His MMA levels range between 20 and 50 M. At 6 months of age, he had mild hypotonia. Complementation studies and propionate uptake on skin fibroblasts were consistent with *mut^0* status in both cases. **Conclusion:** Though *mut^0* MMA is classically Cbl unresponsive, our patients have shown acceptable and stable MMA levels while reciving Cbl supplementation. The mechanism for this responsiveness is unclear at this time. Mutation analysis may help to shed some light on this phenomenon.

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An overview of ophthalmologic findings seen in Cobalamin C deficiency. *P. Tanpaiboon[1], B.P. Brooks[2], J. Slaon[1], N. Braverman[3], C.P. Venditti[1].* 1) National Genome Research Institue/NIH, Bethesda, MD; 2) National Eye Institute/NIH, Bethesda, MD; 3) Institute of Genetic Medicine and Dept. of Pediatrics,Johns Hopkins Medical Center,Baltimore, MD.

Cobalamin C deficiency (*cbl*C) is a rare autosomal recessive metabolic disorder of intracellular cobalamin (vitamin B12) metabolism. The defect impairs the synthesis of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) and features methylmalonic acidemia and homocysteinemia. Ophthalmologic findings are varied and include horizontal nystagmus; decrease visual acuity; macular pigment abnormalities, often with a bulls eye appearance; progressive peripheral pigmentary retinopathy; and optic nerve atrophy. The evolution of the eye lesions is unknown. Seven patients with *cbl*C defect, including two sets of sibs where the index case was diagnosed symptomatically, underwent dilated eye examination and retinal photography in addition to metabolic studies. All seven patients had macular pigmentary abnormalities and/or atrophy. Four patients had strabismus and two had significant refractive errors. Two patients had optic atrophy. One patient treated prenatally and one treated presymptomatically appeared to have a milder ocular phenotype than their sibs; prenatal treatment, however, did not halt the macular changes. One patient had dysmorphic maculae indicating that a prenatal component may underlie the optic pathology in a subset of patients. The oldest individual (age 12) previously had macular changes similar to those observed in younger patients, but progressed to develop pan-retinal pathology and optic atrophy. These observations suggest that the metabolic derangement affects all areas of the retina and may have a defined evolution which first involves the macula, then the periphery and later the optic nerve. A large collection of retinal photographs with clinical correlations may help generate a staging schema for the eye lesions seen in this disorder and provide a marker of disease evolution.

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Identification of Propionate Metabolic Genes in C. elegans: Functional Characterization of Methylmalonyl-CoA Mutase. *M. Tsai[1], R.J. Chandler[1], V. Aswani[1,2], M. Falk[3], M. Sedensky[3,4], P. Morgan[3,4,5], C.P. Venditti[1].* 1) GDRB, NHGRI, NIH, Bethesda, MD; 2) Marshfield Clinic, Marsfield WI; 3) Dept. of Genetics, CASE School of Medicine; 4) Dept. of Anesthesiology, University Hospitals of Cleveland; 5) Dept. of Pharmacology, CASE School of Medicine.

Methylmalonic acidemia is an autosomal recessive inborn error of metabolism caused by a defect in the conversion of L-methylmalonyl-CoA to succinyl-CoA. Its etiology is heterogenous, due to either defects in the apoenzyme or impaired cofactor synthesis. In an attempt to develop a tractable experimental system to study this group of conditions, we have performed genomic and biochemical characterization of the putative propionate to succinate conversion pathway in C. elegans. Homologues of PCCA, PCCB, MMAA, MMAB, MCM, and MMCR, which are involved in propionate metabolism in humans, were identified in C. elegans and cloned for functional studies. Loading of C. elegans with propionic acid caused the animals to produce methylmalonic acid and secrete propionylcarnitine. A variable biochemical response to RNA interference was seen. To further examine the functional properties of the C. elegans methylmalonyl-CoA mutase enzyme, the protein was over-expressed in yeast and kinetic analysis was performed. C. elegans mitochondrial preparations were studied in parallel to demonstrate native enzymatic activity and verify the observed kinetic constants obtained in the yeast system. The native enzyme is present as >70% apoenzyme, with the remainder existing as holoenzyme, indicating that C. elegans makes functional enzyme as well as adenosylcobalamin. Retroviral correction of cells derived from muto mice using the C. elegans MCM cDNA was similar to that achieved with the murine gene. In toto, the combined findings suggest that C. elegans has an active methylmalonyl-CoA mutase and can synthesize adenosylcobalamin, possibly utilizing the same pathways that mammalian cells do. This biological system might be used to identify unknown genes that are mutated in selected cobalamin disorders and to create a model of methylmalonic acidemia amenable to high-throughput genetic and genomic analysis.

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The spectrum of methylmalonic acidemia detected by MS/MS newborn screening in North Carolina. *A. Basinger, S. Pendyal, D. Frazier, J. Koepke, M. Gucsavas-Calikoglu, J. Muenzer.* Department of Pediatrics, University of North Carolina, Chapel Hill, NC.

Since 1997, North Carolina (NC) has screened >900,000 newborn infants using tandem mass spectrometry. Ten cases of methylmalonic acidemia were identified after confirmatory testing of neonates who had both an elevated C3-carnitine (CN) and C3:C2-CN ratio. These fall into three distinct groups. Three African-American infants had mut^0 methylmalonic acidemia (group I), 2 Hispanic infants had mild, persistent elevation of methylmalonic acid (MMA) in both blood and urine without elevation of homocysteine (HCY) (group II), and 5 had both elevated MMA and HCY (group III). The group I patients all presented clinically in the first week of life. They have had typical courses involving multiple hospitalizations for acidosis, poor feeding, neutropenia, and delayed development. The group II patients are both healthy, receive no supplementation, are on unrestricted diets, and are being monitored. Of the five group III patients, none were B-12 deficient. Four had complementation studies consistent with cobalamin (cbl) C defect. A Caucasian female developed lethargy, poor feeding, and hypothermia on day of life 11 and died on day of life 21 from intracranial hemorrhage. A Caucasian male with hydrocephalus died at 5 months from a pulmonary hemorrhage. Three Hispanic children have milder disease and are developing normally with treatment. None have had acidosis or decompensation with illness. Treatment has included 1 mg intramuscular (IM) hydroxycobalamin 2-3 times per week and mild protein restriction using a metabolic formula. They have had normalization of HCY and reduction of MMA to near normal. The siblings of one of these three Hispanic patients were recently tested, and a 9 year old asymptomatic sister with normal development was found to be affected. A trial of oral hydroxycobalamin in this mildly affected patient did not lower her HCY or MMA. The subgroup of patients with cbl C defect and a mild presentation may represent a mutation which is more prevalent in the NC Hispanic population.

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Modeling central nervous system alterations in human glycerol kinase deficiency (GKD) using the glycerol kinase (Gyk) knockout (KO) mouse. *N. MacLennan[1], C. Shin[1], L. Kim[1], L. Rahib[2], K.M. Dipple[1,2,3], E.R.B. McCabe[1,3].* 1) Pediatrics, UCLA, Los Angeles, CA; 2) Biomedical Engineering, UCLA, Los Angeles, CA; 3) Human Genetics, UCLA, Los Angeles, CA.

GKD is an X-linked inborn error of metabolism associated with central nervous system (CNS) deterioration. We showed previously that the Gyk KO mice accurately model the human phenotype. The purpose of these investigations was to elucidate alterations in mRNA expression in brain compared to other tissues in Gyk KOs. We performed microarray analyses on brain, liver, kidney and brown fat mRNA and modeled genes with altered expression using pathway analysis. Gyk gene expression was significantly down-regulated in all tissues. In brain, kinase activity and receptor signaling protein activity were the most significantly upregulated gene functional categories ($p<0.01$), while in liver, kidney and brown fat, the gene groups most significantly upregulated related to metabolism. In all four tissues, the gene groups most significantly downregulated were cell cycle and proliferation, reflecting the severity of the phenotype. Brain KO samples clustered together and away from brain wildtype (WT) samples in both unsupervised and supervised clustering. In unsupervised clustering, the brain was the most different of the tissues. An elaborate network of genes specifically altered in KO brain microarray data were identified by pathway analysis. Notable genes included midkine (-1.7 of WT), stimulated by retinoic acid 13 (-1.4 of WT) and calreticulin (-1.5 of WT), all believed to play a role in cell survival in developing brain. Calreticulin interacts with the DNA-binding domain of the glucocorticoid receptor and prevents receptor binding which is intriguing given GK's involvement in nuclear uptake of the glucocorticoid receptor. In conclusion, Gyk KO brain has a different pattern of altered gene functional categories and gene expression compared with liver, kidney and brown fat. These alterations in brain along with Gyk ablation may contribute to the CNS deterioration in GKD. Further studies of Gyk's role in murine brain development will provide critical insights into mechanisms of CNS deterioration in GKD.

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Free radicals and antioxidant defenses in X-linked adrenoleukodystrophy patients. *M. Deon[1,3], A. Barschak[1,3], A. Sitta[1,3], S. Landgraff[1], G. Oliveira[1], T. Terroso[1], M.H. Oliveira[1], D.M. Coelho[1], M. Wajner[1,3], L. Jardim[1], R. Giugliani[1,3], C.R. Vargas[1,2].* 1) Medical Genetics Service, HCPA, Porto Alegre, Brazil; 2) Dep. of Clinical Analysis, Pharmacy Faculty, UFRGS, Porto Alegre, Brasil; 3) Dep. of Biochemistry, ICBS, UFRGS, Porto Alegre, Brazil.

X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder, biochemically characterized by the accumulation of very long chain fatty acids (VLCFA), particularly hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0) in tissues and biological fluids. Patients affectted by this disorder present predominantly central and peripheral demyelination in addition to adrenal insufficiency. The mechanisms underlying the brain damage in X-ALD are poorly known. Considering that free radical generation is involved in various neurodegererative disorders, like Parkinson disease, multiple sclerosis and Alzheimer's disease, in the present study we evaluated some oxidative stress parameters, namely tiobarbituric acid reactive species (TBA-RS) and total antioxidant reactivity (TAR) in plasma of X-ALD symptomatic patients. It was verified a significant increase of plasma TBA-RS, reflecting induction of lipid peroxidation, as well as a decrease of plasma TAR, indicating a deficient capacity to rapidly handle an increase of reactive species. Therefore, we may conclude that oxidative stress contributes at least in part to the pronounced neurological dysfunction found in X-ALD. In this scenario, it has been proposed that the neurological damage that occurs in X-ALD could be mediated by astrocyte activation and cytokines pro-inflammatory induction. Since free radicals are also involved in inflammatory response, our findings may reflect the same phenomenon. It is therefore proposed that oxidative stress may be involved in pathophysiology of X-ALD. Finally, since so far no therapy for X-ALD symptomatic patients has been proven to be successful, it may also be presumed that the administration of antioxidants should be considered as a potential therapy for the patients affected by X-ALD.

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Genomics of Glycogen Storage Disease type Ia (GSDIa):Global gene expression analysis in glucose-treated and untreated glucose-6-phosphatase knock-out mice. *T.V. Damodaran, A. Schneider, A. Bird, Y.-T. Chen, D.D. Koeberl.*
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GSDIa (Von Gierke Disease), is caused by a deficiency of glucose 6-phosphatase (G6Pase) activity and accompanying glycogen accumulation in the liver and kidney. Clinical manifestations include growth retardation, hypoglycemia, and increased risk for hepatocellular carcinoma. G6Pase knockout (G6Pase-KO) mice (Gift from J.Y. Chou, NICHD) showed all of the clinical characteristics of GSD-Ia. Using this model, we explored the global gene expression changes in glucose-treated (GT) and untreated (UT) G6Pase-KO mice. We hypothesized that pathway-specific microarrays concurrently might help us to identify key glucose-response and G6Pase system-response genes, including: A) Insulin, B) Signal transduction, C) Cell cycle, and D) Apoptosis (Superarray Biosciences, Bethesda, MD). G6Pase-KO (-/-) mice and wild-type siblings (+/+) were analyzed at 10 days old. Affected (-/-) mice were treated with 10% glucose, 1 gm/kg twice a day, which was stopped at 7 days old for UT affected (-/-) mice. RNA from liver, kidney, brain and heart was analyzed, and validation was performed for selected genes from each pathway that confirmed the gene expression patterns identified. Salient findings include the following: A) The expression profiles were distinctly different for GT and UT groups for all tissues studied; B) Liver and kidney showed similar expression profiles in GT mice, and frequently showed opposite trends from UT mice; C) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was over-expressed in both GT and UT groups, and represented a potential biomarker for GSDIa. Increased GAPDH may be involved in non-enzymatic moonlighting activities such as transcriptional regulation, thereby playing major role in pathogenesis; D) Alterations related to increased incidence of malignancy in GSD1A liver included altered expression of genes involved in cell cycle control (eg. Ccne1, Ccnd1). Pathway-targeted microarray analysis of GSDIa mice revealed biomarkers that will be informative regarding pathogenesis, responsiveness to experimental therapies, and potential screening strategies for GSDIa in humans.

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Mitochondrial disease genetics: mito-myths and mito-facts in 329 children with definite respiratory chain disorders. *D.R. Thorburn, A. Boneh, S. Walsh, E. Oldaker, D. DuSart, M. Smith, H.H. Dahl, D.M. Kirby.* Murdoch Childrens Research Institute and Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, VIC, Australia.

We are referred almost all suspected cases of childhood-onset mitochondrial disease (minimum prevalence 6.2/100,000) in Australia and New Zealand. We sought to highlight a number of common misconceptions that may impede optimal investigation and management. We performed a retrospective review of records of 329 children diagnosed by our centre with definite respiratory chain defects since 1990. Results: Histochemical investigations were diagnostic in less than 10% of children. Most diagnoses are based on finding a respiratory chain enzyme defect in a skeletal muscle biopsy. However, over 40 patients, mostly with hepatoencephalopathies, had nondiagnostic muscle investigations with the diagnosis based on liver enzymology. Pathogenic mitochondrial DNA (mtDNA) mutations were identified in 84 patients from 72 families (14 mtDNA rearrangements, 29 MTTL1, 1 MTTK, 21 MTATP6, 3 MTND1, 4 MTND3, 5 MTND5, 7 MTND6); the most common diagnoses being Leigh disease, MELAS and CPEO. A family history suggesting maternal inheritance was the exception rather than the norm and many of the children had de novo mtDNA mutations. Pathogenic nuclear gene mutations were identified in 40 patients (31 families) in 9 different genes (2 NDUFS2, 1 NDUFS4, 3 NDUFS6, 2 NDUFV1, 16 SURF1, 1 SCO2, 7 POLG, 2 DGUOK, 6 TAZ) causing complex I, complex IV or combined enzyme defects. In these patients the most common diagnoses were Leigh disease, Lethal Infantile Mitochondrial Disease and Alpers disease. Enzyme-based or molecular prenatal diagnosis was performed in ~50 pregnancies at risk for nuclear or mtDNA mutations and 7 affected pregnancies were identified. Conclusion: The approach to diagnosis of mitochondrial disease in children varies depending on clinical presentation. We achieved a molecular diagnosis in 38% of patients but most children require muscle and/or liver biopsy to obtain an enzyme diagnosis and to guide molecular analysis of the 34 mtDNA and 41 nuclear genes with pathogenic mutations identified to date.

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New strategies for the rapid identification and quantification of mitochondrial DNA mutations in patients with respiratory chain defects. *V. Procaccio[1,2], S. Bannwarth[3], V. Paquis-Flucklinger[3,4], N. Neckelmann[5], R. Jimenez[1], A. Davila[1], J. Poole[1], M. Simon[1], D.C. Wallace[1,2].* 1) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, USA; 2) Department of Pediatrics, University of California, Irvine, USA; 3) Department of Medical Genetics, Archet 2 Hospital, CHU Nice, France; 4) FRE CNRS/UNSA 2720, Medicine School, University of Nice-Sophia Antipolis, France; 5) Transgenomic, Inc. Omaha, NE, USA.

Molecular analysis of mitochondrial DNA (mtDNA) is a critical step in the diagnosis of mitochondrial disorders. We are currently developing a two-tier strategy for the comprehensive analysis of the entire mitochondrial genome. First, we score for the presence of the most common heteroplasmic and homoplasmic mutations that are known to be pathogenic. Scoring is accomplished by using an innovative and sensitive HPLC-mediated restriction fragment analysis process. This method does not only detect the presence of specific mutations, but also quantifies the relative percentage of mutant DNA in the sample. Mutations at levels as low as 1% can be detected and quantified. If none of the specific known mutations are found at this stage we revert to a rapid scanning procedure that is designed to detect unknown and known heteroplasmic mutations. The scanning method is based on the use of a new mismatch-specific endonuclease known as Surveyor Nuclease.We have shown that in heteroplasmic samples known and unknown mutations can be detected at levels as low as 3%. These methodologies have been used to detect mtDNA mutations in DNA samples extracted from patients blood. Using these approaches we have been able to discover mutations that have not been reported previously or because conventional techniques failed to detect them. We anticipate that positive mutation detection in blood or other tissues may avoid the need for invasive muscle biopsies in numerous cases, and will enhance our ability to detect low levels of mtDNA mutations in potential carriers, crucial for genetic counseling.

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The mitochondrial DNA point mutation at the discriminator base of tRNA-Glutamate is necessary to cause benign infantile cytochrome c oxidase deficiency. *M. Mimaki[1,2], H. Komaki[1], Y. Kirino[3], T. Suzuki[3], Y. Goto[1].* 1) Mental Retardation/Birth Defec, National Inst Neurosci, Tokyo, Japan; 2) Department of Pediatrics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Tokyo, Japan.

Benign infantile cytochrome c oxidase (COX) deficiency is distinct because of its unusual course with clinical and pathological improvement. We experienced 6 patients with this disease, which is characterized by weakness, feeding and respiratory difficulties, mitochondrial myopathy, and lactic acidosis. Only skeletal muscle appears to be affected, and this condition improves spontaneously at 6 to 9 months of age. COX activity is severely reduced in skeletal muscle, but return to a normal level after 1 to 3 years. This disease was thought to be attributed to a nuclear DNA mutation, however, we identified T14674C or T14674G homoplasmic mutation in the mitochondrial DNA in all of the 6 patients. It is located at the end of the aminoacyl acceptor stem of the tRNA-Glu, which plays an important role in tRNA identity as the discriminator base. The nucleotide changes were not found in 200 normal individuals. Partial RNase T1digestion of total RNA from the patient confirmed the mutation in the tRNA itself corresponding to the DNA mutation. Although acid PAGE analysis of RNA extracted from myoblasts of the patient showed normal aminoacylation of tRNA-Glu, northern blot analysis using muscle specimens revealed reduction of tRNA-Glu molecules as compared with normal infants. From these facts, we think the mutation at nucleotide position 14674 is essential cause of this disease. Early differential diagnosis between fatal and benign infantile COX deficiency by detection of this mutation is critically important for management of these infants, because the benign form is initially life-threatening but reversible. However, the mechanism of the recovery remains to be explained. A developmental change in physiological demand of tRNA-Glu quantity or a switch of enzyme from the defective fetal isoform to a normal adult one could account for the improvement.

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Histological Abnormalities and Mitochondrial Dysfunction in *Klotho* and Natural Aging Mice. *K. Hirata[1,2,3], Y. Akita[2,3], Y. Koga[2,3].* 1) Department of Living Environment, Saga Junior College, Saga, Japan; 2) Pediatrics and Child Health, Kurume University School of Medicine, Kurume, Fukuoka, Japan; 3) Kurume University, Cognitive and Molecular Research Institute, Kurume, Fukuoka, Japan.

Klotho (Kl) mouse is known as a premature aging model showing a short lifespan, infertility, arteriosclerosis, hypogonadism, skin atrophy, osteoporosis, emphysema, and growth retardation including sexual development at 3-4 weeks of age to expire at 8-9 weeks of age. Kl gene products express especially in kidney and brain. Kl mouse induce abnormal calcium metabolism to cause notable calcification in kidney, however we still do not know the precise mechanism. We focused in kidney and cerebrum to analyze histological properties and the mitochondrial function such as respiratory chain enzyme activities and oxygen consumption in mitochondrial and synaptosomal fractions to examine whether Kl mice show the same properties as natural aging mice. We performed the biochemical analysis of natural aging (CD-1, averaged 84 weeks of age) and Kl (7 weeks of age) mice. The impaired mitochondrial respirations and histological abnormalities in Kl mice bore some resemblance to natural aging mice. The mitochondrial impairment by abnormal calcium metabolism of Kl mice induced senescence, as natural aging mice, but the histomorphometrical changes and the some changes of respiratory enzyme activities in kidney and cerebrum were different with natural aging mice. We elucidated that the defect of Kl gene expression in Kl mice cause the abnormal mitochondrial energy metabolism in both tissue and the histological abnormalities with some differences such as retardation of cerebral development and abnormalities of cerebral synaptic vesicles in Kl mice between Kl and natural aging mice. It is suggested that the defect of Kl gene expression induce the abnormal mitochondrial homeostatic system to make the developmental disabilities in cerebrum and kidney, and the sign of senescence for Kl mouse.

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A new mitochondrial DNA mutation in ND3 gene is associated with isolated complex I deficiency and Leigh syndrome in 3 independent families. *E. SARZI[1], D. CHRETIEN[1], S. LEBON[1], A. ROTIG[1], A. MUNNICH[1], M. BROWN[2], V. PROCACCIO[3].* 1) INSERM U393, Genetics Department Necker Hospital, Paris, France; 2) Mercer University School of Medicine, Macon, GA, USA; 3) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, USA.

NADH:ubiquinone oxidoreductase (complex I) is the largest complex of the mitochondrial respiratory chain and this complex accounts for most cases of respiratory chain deficiency in human. Complex I contains at least 46 subunits and 7 of which are encoded by mitochondrial DNA (mtDNA). Previous studies have shown that at least 20% of respiratory chain complex I deficiencies are related to mtDNA mutations. Here we report three unrelated families with Leighs syndrome (LS). The mother of the first family of French origin reported difficulties in walking and headaches. She had 3 children, who developed in the first weeks of age, LS associated with a complex I deficiency identified in muscle or liver. Sequence analysis of mitochondrial complex I genes revealed an homoplasmic G to A mutation at nt G10197A in ND3 gene in patient muscle DNAs. The mothers leukocytes showed 50% of heteroplasmy. Two patients from a second family of French origin also presented LS and a complex I deficiency in liver and fibroblasts. The G10197A mutation was found to be homoplasmic in those tissues whereas the asymptomatic mother presented 70% of mutant mtDNA molecules in leukocytes. The mother of the third family was affected with generalized dystonia with childhood onset and bilateral basal ganglia lesions. She had 3 affected children with LS. An isolated complex I deficiency was found in muscle associated with the heteroplasmic G10197A mutation. The G10197A mutation modifies a hydrophobic amino acid alanine residue into a hydrophilic threonine (A47T) in a highly conserved part of ND3 subunit. The discovery in 3 unrelated families of the G10197A mutation with a similar phenotype establishes the pathogenicity and demonstrates that the A47T is important for the function of complex I.

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Natural history of mild elevations of C5OH detected by newborn screening. *G.L. Arnold, E.M. Howell, L.R. Mead.*
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Introduction: Newborn screening (NBS) by TMS was recently expanded in New York State to include 3-Methylcrotonyl CoA Carboxylase (3MCC) deficiency using the marker C5OH. In New York a C5OH level 0.6 molar triggers an urgent referral to a metabolic center.

Methods: We reviewed the clinic charts of all 11 patients referred by the state NBS program for critical elevations of C5OH in the first 6 months of the expanded program.

Results: Four infants were pre-term (range 29-36.5 weeks) and seven were term; mean gestational age (GA) was 36.8 weeks. Median age at initial screen was 2 days (range 1-16). Mean birthweight (BW) was 2960 gm (range 1415-3816). Mean initial NBS C5OH level was 1.03 molar (range 0.79-2.54). Initial C5OH level was not correlated with GA, BW, or the age at NBS.

All infants are clinically well. Follow-up acylcarnitine and urine organic acid (OA) analyses were performed at the initial clinic visit. On follow-up six infants now had C5OH levels within the reference range for the laboratory (0.09) and normal OA; no further testing was done. Of the remaining 5 infants the follow-up C5OH level was 0.16-0.4. Two infants had normal OA and C5OH levels of 0.23-0.3 (including one whose mother was found to have a mild persistent elevation of C5OH with normal plasma carnitine levels); no further testing was done. Two others had mild elevations of 3-isovalerylcarnitine and 3-methylcrotonylglycine on OA analysis. On serial testing one of these infants normalized the C5OH level by month 2. However at age 4 months one remains with mild elevation (0.39); this is also the infant with the highest NBS C5OH level of 2.4 molar.

Discussion: Mild elevation of C5OH can be found in both pre-term and healthy term newborns; most have normal OA and the C5OH elevations usually resolve within weeks. Infants having low-grade abnormal metabolites on urine OA analysis may take longer to resolve blood C5OH levels or may have persistent mild elevations of unclear significance.

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Whole blood and leukocyte intracellular glutathione levels are low in organic acidemias. *G.M. Enns[1], K.R. Atkuri[2], T.M. Cowan[1,3], S.M. Huegenin[3], M. Lyons[1], L.A. Herzenberg[2], L.A. Herzenberg[2].* 1) Dept. Pediatrics, Divs Med. Genet; 2) Dept. Genetics; 3) Dept. Pathology, Stanford University, Stanford, CA.

Primary disorders of organic acid metabolism may cause secondary mitochondrial dysfunction, leading to increased production of intracellular reactive oxygen species (iROS) and depletion of intracellular antioxidants. The glutathione system is the main natural defense mechanism that helps to combat oxidative damage, but it has not been studied systematically in individuals with organic acidemias. In this report, we analyzed intracellular and whole blood glutathione levels in three patients with methylmalonic acidemia (*mut*^{0}) and one with propionic acidemia using Hi-D fluorescence activated cell sorting (FACS) and tandem mass spectrometry (MS/MS). Peripheral blood buffy coats were treated with monochlorobimane (MCB), then stained for leukocyte subset markers and subjected to FACS. When compared to normal controls (n=27), iGSH levels in CD4 (MCB ratio 1.12 $\pm$ 0.15 v. 1.66 $\pm$ 0.27 in controls, p=0.002) and CD8 (MCB ratio 1.04 $\pm$ 0.20 v. 1.52 $\pm$ 0.21 in controls, p=0.003) T lymphocyte subsets and neutrophils (MCB ratio 2.15 $\pm$ 0.85 v. 2.65 $\pm$ 0.61, p=0.044) were significantly lower in subjects with organic acidemias. iGSH levels tended to be lower in monocytes (p=0.054), but a significant difference in B cells was not observed. Consistent with FACS results, the mean whole blood glutathione level in organic acidemia patients was 60% of normal controls when analyzed by MS/MS. Immunodeficiency, in addition to neutropenia, has been reported in patients with organic acidemias. The pathogenesis of immune dysfunction in organic acidemia patients may in part be explained by low leukocyte iGSH levels leading to damage of specific immune cell subsets. Because N-acetylcysteine (NAC) restores iGSH levels by providing the cysteine necessary for its biosynthesis, NAC therapy may be beneficial in both acute and chronic treatment of organic acidemias.

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Characterization of two patients with mut^0 methylmalonic acidemia after combined liver-kidney transplantation: failure to normalize CSF metabolism. *J.L. Sloan[1], M. Merideth[1], T. Bunchman[2], J.J. Gargus[3], R. Koch[4], C.P. Venditti[1].* 1) NHGRI, NIH, Bethesda, MD; 2) DeVos Children's Hospital, Grand Rapids, MI; 3) UCI, Irvine, CA; 4) Childrens Hospital LA, Los Angeles, CA.

Methylmalonic acidemia (MMAemia), caused by deficiency of the methylmalonyl CoA mutase is a devastating disorder, recalcitrant to medical management in many cases. Several patients have received kidney, liver and combined liver-kidney transplantation as a treatment, with mixed results. We describe the metabolic phenotype of two patients with mut^0 class MMAemia s/p liver and kidney transplantation who were studied in the inpatient setting. Case 1, a 19 year-old female, was diagnosed at 2 days following an episode of hypothermia and lethargy. At age 12, she received a cadaveric renal transplant and subhepatic heterotopic auxiliary liver transplant. Case 2, a 27 year-old female, was diagnosed in the neonatal period. At age 22, she received an orthotopic liver transplant, followed by a kidney transplant two years later. Transplantation has protected the patients from ketoacidotic crises. Both have persistent MMAuria, range 1197-2266 mg/gCr (nl<3). Other parameters were essentially identical, including plasma MMA concentration (260-270 M) [nl<0.27] and whole body MMA output (14 mg MMA/kg/d). Case 2 demonstrated reduced MMA output and decreased plasma MMA levels (268 M vs. 181 M) with dietary protein restriction. The concentration of MMA in the CSF of both patients was >500 M [nl<0.59], more than two-fold that of the plasma MMA. 2-methylcitrate was also increased in the CSF > plasma. Creatinine clearance was normal. CONCLUSIONS: Persistent elevations of MMA and 2-methycitrate and modulation by precursor intake indicate that combined liver-kidney transplantation is not curative for MMAemia. High levels of MMA in the CSF s/p LKT may increase the risk to develop a metabolic stroke or CNS dysfunction. The possibility of further renal injury post-transplant must also be considered given the massive MMAemia/uria. Nutritional control and CSF monitoring may be required for optimal management. Further studies on the post-transplant state are needed, particularly on outcome and metabolic characterization.

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Characterization and identification of mitochondrial cobalamin binding proteins in patients with methylmalonic aciduria. *E. Moras[1,2], D. Watkins[2], D.S. Rosenblatt[1,2].* 1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada.

Vitamin B_{12} (Cbl) is necessary for the function of two human enzymes: methylmalonyl CoA mutase (MCM) and methionine synthase (MS). The mitochondrial enzyme, MCM, catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA and uses adenosylcobalamin (AdoCbl) as a cofactor. Defective MCM enzyme (complementation group *mut*) or a deficiency in the synthesis or utilization of AdoCbl (*cblA*, *cblB*, and *cblH*) results in methylmalonic aciduria (MMA). In the cytosol, methylcobalamin is utilized as a cofactor for MS in the conversion of homocysteine to methionine. The *cblC*, *cblD*, and *cblF* disorders result from defects in the synthesis of both cofactors and therefore present with combined MMA and homocystinuria. In man, Cbl has been shown to bind to MCM in mitochondria and to MS in the cystosol. Using fibroblast cell lines from patients with different inborn errors in AdoCbl synthesis, we attempted to determine if other mitochondrial Cbl binding proteins involved in AdoCbl synthesis and/or metabolism exist. We also tried to understand how known mutations in these patients might affect Cbl binding to accessory proteins. Fibroblasts were incubated with [^{57}Co]CNCbl and mitochondrial fractions were obtained. Using gel filtration chromatography, proteins were separated based on molecular weight. In control cells, the majority of radioactivity (65%) eluted with an apparent molecular weight of 150kD, corresponding to MCM. Unbound Cbl (1355D) was also identified (16.2 %). Six per cent of the radiolabelled Cbl eluted with a previously unidentified protein having an apparent molecular weight of 30kD. Extracts from *cblA* fibroblasts showed a Cbl binding profile similar to that of controls. In the *cblB*, *cblH*, *mut* and *cblC* complementation groups, the unidentified peak represented a large amount of the radiolabelled Cbl (28%, 41%, 54% and 73% respectively). These results indicate the presence of an as yet unknown Cbl-binding protein in the mitochondria of human fibroblasts.

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Overexpression of Mouse MethylmalonylCoA Mutase in Yeast: Use to Study Interallelic Complementation and Kinetic Characterization of a Novel Km Mutant. *V. Aswani[1,2], R. Chandler[1], L. Worgan[3], D. Rosenblatt[3], C.P. Venditti[1].* 1) NIH, NHGRI, Bethesda, MD; 2) Marshfield Clinic, Marshfield, WI; 3) Dept of Human Genetics, McGill University.

Studies were undertaken to examine the kinetic properties of the murine MCM enzyme and a series of site-directed mutants that changed homologous amino acids seen in various patients with mut-class MMA, including two that participate in interallelic complementation and one associated with partial impairment of enzymatic function. The R93H, G717V, and G642R mutations were introduced into the mouse gene at homologous positions. The G642R change was identified in a patient with residual propionate incorporation and a mild infantile course. The mutations were cloned under the control of the GAL4 promoter with either URA or TRP markers and introduced into yeast with single or double auxotrophic selection. Antibodies against the mouse protein were generated and used to verify galactose-regulated expression. Activity was measured with the succinate thiokinase linked assay. Km and Vmax parameters and their distribution-free confidence limits were determined by direct linear plots. Robust activity exceeding 2000 nmol/mg/hr was routinely achieved in the yeast extracts, allowing kinetic studies to be conducted with ~15 ml of log-phase yeast as starting material. The WT murine enzyme had native properties when over-expressed and was entirely apoenzyme. The G640R change produced a pure cobalamin Km effect, approximately 10 fold increased vs WT, milder than that previously reported for the human G717V mutation, and may be suitable for expression in the murine model to produce a mut- state. The R91H/G715V double mutant possessed mixed kinetic parameters, intermediate between wild type and the individual mutants, demonstrating that interallelic complementation can be replicated in yeast by co-expression of two mutant subunits. Detailed kinetic analysis has also allowed cofactor administration in the affected patient with the G642R mutation to be reconsidered. The natural mutations seen in patients with mut class MMA should be studied in greater detail to allow genotype-enzymatic correlations to be examined.

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The molecular basis of atypical methylmalonic aciduria: Search for mutations in the methylmalonyl-CoA mutase and methylmalonyl-CoA epimerase genes. *A.B. Gradinger[1,3], L.C. Worgan[3], J.P. Lerner-Ellis[1,3], D. Watkins[3], E. Moras[1,3], D. Roquis[2], C. Doré[2], D.S. Rosenblatt[1,3].* 1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada; 3) Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada.

The methylmalonic acidurias occur due to a defect in methylmalonyl-CoA mutase (MCM) (*mut*) or due to inborn errors in the metabolism of vitamin B_{12} (cobalamin) that result in a block of the synthesis of adenosylcobalamin (AdoCbl) (*cblA*, *cblB*, *cblC*, *cblD*, *cblF*, or *cblH*). D-methylmalonyl-CoA is generated during the catabolism of branched chain amino acids, odd chain fatty acids and cholesterol. Methylmalonyl-CoA epimerase racemizes D-methylmalonyl-CoA to L-methylmalonyl-CoA, which is then converted to succinyl CoA by MCM, using AdoCbl as a cofactor. It has been suggested that defects in epimerase will also result in methylmalonic aciduria (MMA), but no confirmed cases of epimerase deficiency have been identified. We have sequenced the *MUT* and *MCEE* genes (coding for MCM and epimerase respectively) from 30 patients with mild MMA, who could not be assigned to any of the known disorders causing MMA. Their cultured fibroblasts showed reduced incorporation of labeled propionate into cellular macromolecules (a measure of functional MCM activity). Patients ranged from being clinically unaffected to having severe presentations such as muscular hypotonia, acidotic crises and developmental delay. Analysis of the *MUT* gene revealed a heterozygous missense mutation in one patient (c.1091A>C, p.Y364S). As well, a heterozygous silent base change (c.711A>G, p.P237P) was present in two patients. Neither of these changes was seen in 48 CEPH controls. Sequencing of the *MCEE* gene showed two patients to be homozygous for a nonsense mutation (c.139C>T, p.R47X). Another patient was shown to be heterozygous for a deletion in the 3 untranslated region (c.*+120_121delTG). Neither of these changes was seen in 70 CEPH controls. Thus, in this subset of patients, it is possible that the changes found in the *MUT* or *MCEE* genes contribute to their mild MMA.

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Combined methylmalonic and malonic aciduria with cerebral organic acidosis in a 52 year old man. *K. O'Brien[1], J. Sloan[1], C. Whitley[2], I. Bernardini[1], A. Gropman[3], C. Venditti[1].* 1) NHGRI/NIH,Bethesda,MD; 2) University of MN,Minneapolis,MN; 3) Georgetown University,Washington, DC.

Combined methylmalonic and malonic aciduria (CMAMMA is a rare metabolic disorder with an unknown etiology. Metabolic findings include increased malonic acid (MA) and methylmalonic acid (MMA) in the urine. We describe the case of a 52 year old white male with progressive neuropsychiatric complications, no history of metabolic decompensation, and methylmalonic acidemia. Over an eight year period he developed progressive cognitive decline with labile moods, and began experiencing seizure-like episodes. He was treated for a seizure disorder based on his history and an abnormal baseline electroencephalogram (EEG), but did not respond to anti-epileptic therapy. Intramuscular hydroxycobalamin failed to lower his plasma MMA level or improve symptoms. Neurocognitive assessment revealed memory loss, attentional difficulties, motor asymmetry, a sensory neuropathy, and bilateral coordination deficits. Magnetic resonance imaging of the brain demonstrated a right choroid plexus cyst and punctuate lesions in the cerebellar hemispheres. Magnetic resonance spectroscopy of brain white matter, cortical and deep gray matter, and cerebrospinal fluid (CSF), was unremarkable. Laboratory studies showed an elevated plasma MMA level of 11 uM with normal vitamin B12 and homocysteine levels. Fibroblast MalonylCoA decarboxylase activity was normal. His urine organic acid (OA) profiles demonstrated increased MMA (9-111 mmol/mol Cr, normal <4) and MA (9-16 mmol/mol Cr, normal<2) without other propionate-derived metabolites. The plasma MMA levels ranged between 10.0-15.3 uM. CSF analysis showed increased MMA, MA and detectable ethylmalonic acid, with a CSF to plasma MMA gradient greater than 5-fold. This patient appeared to have the CMAMMA syndrome based on urine OA analysis. However, his CSF organic acid profile is compatible with a combined semialdehyde metabolic perturbation. In some patients the natural history of the CMAMMA syndrome may feature altered central nervous metabolism and a predisposition to neuropsychiatric complications. The natural history of this syndrome needs further delineation.

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Pearson syndrome with abnormal urine organic acids and a novel mitochondrial DNA deletion. *Y. Qu[1], H. Bass[2], M. Jamehdor[1], R. Mardach[2,3].* 1) Dept. of Genetic Testing, Kaiser Permanente, Los Angeles, CA; 2) Dept. of Genetics, Southern California Permanente Medical Group, Los Angeles, CA; 3) Regional Metabolic Service, Kaiser Permanente, Los Angeles, CA.

Pearson marrow-pancreas syndrome is a rare mitochondrial deletion syndrome characterized by refractory anemia with pancreatic dysfunction. The phenotypic presentation is variable depending on the amount of mitochondrial DNA deletion found in different tissues. Without identification of a mutation, the diagnosis can be difficult. We report a patient with Pearson syndrome who had abnormal urine organic acids and a novel mitochondrial DNA deletion.

The patient is the fourth child born prematurely at 35-week gestation to a non-consanguineous couple. Family history was unremarkable. During the neonatal period, the baby was found to have pancytopenia and hepatomegaly. A metabolic work-up revealed normal plasma amino acids and acylcarnitines but aminoaciduria. Urine organic acid (UOA) analysis showed lactic aciduria with ketosis. The most notable excretion of organic acids (in mmol/mol creatinine) are: lactate, 2157 (N=0-150); 2-hydroxybutyrate, 125 (N=0-3); 2-hydroxyisovalerate, 52 (N=0-1); 3-hydroxybutyrate, 565 (N=0-15); 3-hydroxyisobutyrate, 1079 (N=0-75); 3-hydroxypropionate, 934 (N=0-15); propionylglycine, 8 (N=0); fumarate, 201 (N=0-17) and malate, 518 (N=0-62). However, 3-methylglutaconic acid, which may be found in patients with mitochondrial disorders, was within normal range. Branched-chain amino acid in-vitro probe assay was conducted on fibroblasts, and no specific defects were detected. Analysis of mitochondrial DNA revealed a novel 5.2 kb deletion confirming the diagnosis of Pearson syndrome.

Relevant clinical symptoms and the presence of lactic aciduria, ketosis, together with increased excretion of branched-chain amino acid and TCA cycle metabolites, should prompt mitochondrial DNA testing. More data should be collected to analyze if a correlation exists between an abnormal UOA pattern and a mitochondrial DNA mutation on a clinical background.

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A novel quantitative method for detection of allele-specific RNA expression. *E. Klootwijk, E. Gottlieb, C. Ciccone, P.J. Savelkoul, M.S. Sun, D. Krasnewich, W.A. Gahl, M. Huizing.* MGB, NHGRI, NIH, Bethesda, USA.

Hereditary Inclusion Body Myopathy (HIBM) and sialuria are two distinct disorders resulting from mutations in the same gene, *GNE*, coding for the bifunctional, rate-limiting enzyme in sialic acid biosynthesis, i.e., UDP-GlcNAc 2-epimerase (GNE)/ManNAc kinase (MNK). Sialuria is caused by autosomal dominant missense mutations in the allosteric site of GNE/MNK, leading to a loss of feedback-inhibition and increased excretion of sialic acid. In contrast, HIBM is caused by autosomal recessive *GNE* missense mutations outside the allosteric site, resulting in decreased GNE/MNK enzyme activity and decreased production of sialic acid. Both sialuria and HIBM exhibit variable clinical phenotypes. We examined whether there exist mutation-dependent variations in allelic expression of *GNE*, since these could account for the variable disease phenotypes. We developed a real-time RT-PCR method that rapidly and accurately detects and quantifies allele-specific expression. The procedure is based on the use of a combination of two allele-specific fluorescent reporter probes and real-time amplification kinetics. We first tested the validity of each allele-specific assay across a concentration range obtained by mixing cell-free transcribed normal and mutated *GNE* RNA. Each of the assays proved to be accurate and mutation-specific, allowing us to study allelic expression of *GNE*. Next, we applied the assays to RNA obtained from fibroblasts of sialuria or HIBM patients. No patient showed a significant difference in mutation-dependent allelic *GNE* expression, indicating that allelic expression did not cause the variable phenotypes in these patients with sialuria or HIBM. This novel, allele-specific RNA quantifying method is convenient and rapid, and requires minimal concentrations of RNA (<25ng). The procedure is attractive for various applications, including validation of SI-RNA silencing experiments. In fact, we have demonstrated the methods validity for allele-specific RNA gene silencing using SI-RNAs in sialuria fibroblasts. In addition, this method can be employed for studies of X-chromosomal inactivation, genetic imprinting, and epigenetics.

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Use of knockout mouse models to elicit the pathophysiology involved in obesity and hypertension in Bardet-Biedl syndrome. *M.A. Fath[1,3], K. Rahmouni[2], M. Andrews[1,3], C.D. Sigmund[2], V.C. Sheffield[1,3].* 1) Department of Pediatrics, Division of Medical Genetics; 2) Department of Internal Medicine; 3) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetic disorder with the cardinal features of obesity, retinopathy and polydactyly. We have developed mouse BBS models for BBS2 (*Bbs2*$^{-/-}$) BBS4 (*Bbs4*$^{-/-}$) and BBS6 (*Bbs6*$^{-/-}$) whose phenotypes closely resemble that of humans. We sought to use these models to dissect the mechanisms involved in the metabolic and cardiovascular disorders associated with BBS. We found that BBS null mice develop obesity due to both hyperphagia and a decrease in activity. We examined whether the obesity in the BBS null mice is associated with the loss of leptin action or leptin resistance. Leptin is an adipocyte-derived hormone that promotes weight loss by decreasing appetite and increasing energy expenditure. We found high circulating levels of leptin in the BBS null mice. As expected, injections of leptin caused a decrease in body weight and food intake in the wild type mice. Leptin failed to reduce appetite or body weight in both *Bbs2*$^{-/-}$and *Bbs4*$^{-/-}$adult mice but had a modest effect on young animals. This indicates that the hyperleptinemia observed in the BBS null mice increases with age and adiposity. Next we compared arterial pressure between wild type and BBS null mice. *Bbs4*$^{-/-}$and *Bbs6*$^{-/-}$mice exhibited ~12 mmHg higher arterial pressure than their wild type controls. However, *Bbs2*$^{-/-}$mice had normal arterial pressure. Increased sympathetic nerve activity is considered to be an important mechanism of obesity-associated hypertension. Consequently, we recorded the renal sympathetic nerve activity in the knockout mice. *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$mice showed higher baseline renal sympathetic nerve activity as compared to the wild type mice but the *Bbs2*$^{-/-}$mice did not, consistent with the normal arterial pressure findings. All 3 mouse models of BBS exhibit obesity however only 2 exhibit hypertension. This suggests a role for the renal sympathetic nervous system in the high arterial pressure associated with BBS and a unique opportunity to understand the mechanisms of obesity-associated hypertension.

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Genetic disruption of *RanBP2* in the murine impairs glucose catabolism and retinal function. *P. Ferreira[1], A. Aslanukov[1], R. Bhowmick[1], J. Oswald[1], D. Raz[2], R. Bush[2].* 1) Departments of Ophthalmology, Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27110; 2) National Institutes of Health, NIDCD/NEI, Bethesda, MD 20892.

The Ran-binding protein 2 (RanBP2) is a large mosaic protein unique to vertebrates. Selective domains of RanBP2 interact specifically with a diverse set of molecular partners. RanBP2 has been implicated in multiple cellular functions. Yet, the biological relevance and implication/s of these in physiological and pathological processes remain elusive. To probe the function of RanBP2 in animal physiology and disease, we generated a mouse model harboring the disruption of *RanBP2*. Analysis of the Mendelian ratios of the offspring produced from $RanBP2^{+/-}$ x $RanBP2^{+/-}$ crosses supports that $RanBP2^{-/-}$ mice are early embryonic lethal. The *RanBP2* locus was also haploinsufficient. $RanBP2^{+/-}$ mice exhibited a number of physiological phenotypes that were dependent on the age and genetic background. $RanBP2^{+/-}$ males 6 months old in an inbred 129ola, but not in a 129ola/C57Bl6 mixed genetic background, exhibited significant deficits in glucose catabolism upon glucose challenge and when placed on a high fat diet, without impairment of gluconeogenesis and glucose uptake. This was accompanied by a reduction of the levels of hexokinase I (HKI), a partner of RanBP2, and its delocalization in photoreceptors. $RanBP2^{+/-}$ males had decreased body weight beginning at ~4 month of age, but exhibited similar levels of food consumption. $RanBP2^{+/-}$ male mice had ERG deficits. These were manifested by a reduction of the scotopic *b*-wave and a trend towards a reduced photopic *b*-wave and scotopic *a*-wave. Finally, there was no difference in the average of the density of nuclear pores (NPC) at the nuclear envelope (~ 3-4 NPC/m2) between wild type and heterozygous *RanBP2* litters (P0). Altogether, the data support that haploinsufficiency of *RanBP2* causes deficits in glucose catabolism by a RanBP2-mediated reduction in HKI and genetic modifiers modulate this process. Hence, the *RanBP2* mouse model serves as a disease model to complex metabolic disorders such as diabetes type II and allied retinopathies.

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A conditional knockout mouse model for Gaucher disease. *G.B. Sinclair[1], K.E. Colobong[2], F.Y.M. Choy[3], L.A. Clarke[2].* 1) Department ofPathology , Children's and Women's Health Center of British Columbia, Vancouver, Canada; 2) BC Research for Children's and Women's Health, Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Centre for Biomedical Research, Department of Biology, University of Victoria, Victoria, Canada.

Gaucher disease (OMIM 230800), the most common lysososomal storage disorder, results from an inherited deficiency of the enzyme glucocerebrosidase (GBA) (EC 3.2.1.45). Enzyme replacement regimes (ERT) have been effective in altering the disease course in non-neuronopathic (type I) forms. There still remains though a very poor understanding of the pathologic mechanisms underlying the extreme heterogeneity of type I patients, the etiology of neuronopathy in type II and III patients, and the role of ERT across this spectrum. Mouse models for this disease have been attempted using traditional gene knockout, point mutation, and chemical induction approaches, with varying degrees of success. We have utilized Cre and Flpe recombinases in a novel approach to conditionally inactivate the murine GBA locus, allowing for the removal of selectable markers at the ES cell stage, and adding only targeted recombination sites. Non-recombined animals develop normally, while early ubiquitous recombination leads to significant tissue glucocerebroside storage and recapitulates the perinatal lethal phenotype observed with traditional GBA knockout. Hematopoietic and endothelial cell-specific recombination was achieved using the Tie2Cre system. Conditional KO animals are viable but have significant pathology. These animals display 33% of normal GBA activity in the liver, 10-15% in the bone marrow and spleen, and had undetectable activity in peripheral mixed white cells. Tissue glucocerebroside storage was evident with isolated lipid laden "Gaucher" cells in the liver, rafts of these cells in the spleen and measurable splenomegaly by 26 weeks of age. While this current model resembles type I Gaucher disease, this system allows for spatiotemporal control of GBA activity and the modeling of a wide array of Gaucher phenotypes leading to greater understanding of the pathophysiology of disease and further potential therapeutic approaches.

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Linkage analysis in one Chinese family with congenital fibrosis of extraocular muscles and mutation screening of candidate gene. *S. Lu, C. Zhao, N.D. Li, K.X. Zhao.* molecular genetics lab, TianJin Eye Hospital, TianJin, TianJin, China.

Objective To describe the clinical phenotype in a Chinese family with congenital fibrosis of extraocular muscles. To identify the chromosomal location of the disease locus and to sequence the candidate gene in the critical region. Methods The clinical feature of all affected members in this family were examined. A genome-wide linkage screening was conducted. Direct genomic sequencing was used to evaluate the candidate gene KIf21A. Results Four affected members in the pedigree were born with classic phenotype of CFEOM. By linkage analysis the disease gene was mapped to chromosomal region 12p11.2-q12 defined by microsatellite markers D12S1648 and D12S1668. The maximum Lod Score was 2.12 (D12S1090). Direct sequence showed no mutation in all exons and exon-intron boundaries of the candidate gene KIF21A , a polymorphism substitution occurred in the exon 21. Conclusions The disorder in this family should be referred as CFEOM1which was inherited as an autosomal dominant trait. The candidate gene was linked to CFEOM1 locus on chromosome 12p11.2-q12, between marker D12S1648 and D12S1668. It's more likely that KIf21A is not the disease causing gene in this family.

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Nonlinear Transmission/Disequilibrium Test. *M. Xiong[1], J. Zhao[1], E. Boerwinkle[1], C. Amos[2].* 1) Dept Human Genetics, Univ Texas Health Science, Houston, TX; 2) Dept Epidemiology, MD Anderson Cancer Institute, Houston, TX.

With the imminent completion of the International HapMap Project, and the availability of efficient and cost-effective genotyping technologies, genome-wide TDT for linkage and association analysis of complex diseases is feasible. In its original form, the TDT may not optimally reveal information and therefore we introduce nonlinear TDT statistics to amplify the difference in the frequencies of the transmitted and nontransmitted alleles/haplotypes in order to increase statistical power. In this report, we develop theoretical foundations and a general statistical framework for the construction of nonlinear TDT statistics. The type I error rates of the nonlinear TDT statistics in both homogeneous and admixture populations are validated through extensive simulation studies. By analytical methods, we show that several nonlinear TDT statistics have higher power than the original TDT statistics. Using approximation to the noncentrality parameters, we unravel the relationship between the measure of nonlinearity of the nonlinear function and the power of the nonlinear TDT statistic. We also show that a class of similarity measure-based TDT statistics can be derived as nonlinear TDT statistics. Finally, to further evaluate the performance of the nonlinear TDT statistics, the presented nonlinear TDT statistics are applied to two real data examples to test the linkage of the RET gene with Hirschsprung disease the and FC receptor genes with systemic lupus erythematosus. The results show that several nonlinear TDT statistics can reach stringent p-values to ensure genome-wide significance.

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Exploiting Grid Computing for the Analysis of Genetic Linkage Disequilibrium. *W.W.S. Lau, A.R. Collins.* Human Genetics, Southampton General Hospital, Mailpoint 808, Duthie Building, University of Southampton, SOUTHAMPTON, Hampshire, United Kingdom.

Linkage disequilibrium (LD) maps are analogous to linkage maps in that map distances are additive but for disease association mapping they provide potentially much higher resolution. LDMAP is a program for constructing LD maps from single nucleotide polymorphism (SNP) data from large population samples. It employs a sequential algorithm which selects the informative SNP pairwise data for each interval between adjacent SNPs, and models the decline of association with distance centered on each interval. From the HapMap project and other sources, millions of SNP genotypes are now available for constructing the LD maps. However the volume of data imposes a considerable computational load which can be addressed effectively utilizing the computational Grid. Grid computing provides a distributed high-throughput alternative to manage the large scale data processing in which the computing resource is made available on demand transparently. The LDMAP algorithm has been integrated into the Condor system - a cycle stealing scheduler, and has been evaluated on a cluster of more than 1600 nodes. The Grid-Enabled LDMAP has been prototyped, and the LD maps of entire chromosomes are constructed from HapMap samples. An online interface which will allow external users to build maps on remote Grid resources is planned. High resolution LD maps constructed from the high density HapMap samples (3 million SNPs per population projected) will have a substantial impact on the efforts to map complex trait genes and for the study of selection, frequent chromosome rearrangements and other phenomena.

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Merging microsatellite data II: methodology and software to increase power in association studies. *A.P. Presson*[1], *E. Sobel*[2], *J.C. Papp*[2]. 1) Dept Statistics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Correctly merged data sets can increase statistical power in association studies. However, alleles from microsatellite data sets genotyped with different experimental protocols or platforms cannot be accurately matched using base pair size information alone. In a recent publication we introduced MicroMerge, software that merges microsatellite data by matching allele frequencies between data sets. In the current presentation we discuss an alternate algorithm, which produces data sets that can be analyzed by all standard genetic analysis software. The new model considers unique alleles that are observed at only one laboratory to be missing from the other laboratory's data set. This view differs from the previous method that ascribed missing alleles to allele-calling differences, i.e., lumping more than one true allele under a single allele name. In addition to the current, alternate algorithm, we now present extensions of both algorithms to (1) align multiple data sets simultaneously, (2) align data sets that each contain samples from more than one ethnic group, (3) enable user supplied population allele frequency vectors, so that small data sets can be combined, and (4) reject poorly aligned allele pairs instead of entire markers. Finally, we discuss a maximum likelihood approach for assessing alignment quality. These new features extend the application of MicroMerge to a greater variety of data sets and improve the merge yield by preserving all confidently aligned alleles. Preliminary results indicate that the new algorithm successfully aligns data sets, and that the new features will be an asset to the association analysis.

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***In silico* fine-mapping of genetic loci for mammary metastasis in mice.** *P. Liu, H. Vikis, Y. Wang, M. You.*
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Metastasis is a process by which tumors spread from the primary organ of growth to secondary sites in the body. This process is of tremendous clinical importance, since the majority of cancer mortality is associated with metastatic diseases rather than the primary tumors. In this study, we performed a whole-genome linkage disequilibrium (LD) analysis *in silico* for the metastasis efficiency of mammary (MEM) tumors in mice. Genotypic data on 8,868 evenly distributed single nucleotide polymorphisms (SNPs), spanning the mouse genome at an average density of 288kb per SNP, were downloaded from the Novartis Research Foundation (GNF) SNP database. Twenty five inbred strains with different genealogies and distinct metastatic potentials were used for our analysis. We identified two new genomic regions (MEM1 and MEM2) on chromosomes 5 and 9, respectively, which were potentially associated with mammary tumor metastasis (p ¡Ö 0.0006). Potential candidate genes in these two regions were carefully assessed based on previous studies and the gene ontology (GO) inference. We prioritized a small list of candidate genes, including Eif2ak1, Pms2, and Ocm for future confirmation studies. Our study provides important clues toward the identification of the MEM genes.

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Mapping of Autosomal Dominant Progressive Myopathy of a Limb-Girdle Distribution and Bone Fragility to Chromosome 9p21-p22: Identification of a Novel Locus for a Musculoskeletal Syndrome. *G. Watts[1], S. Mehta[1], C. Zhao[2], S. Ramdeen[1], S.J. Hamilton[3], S. Mumm[4], M.P. Whyte[4], B. McGillivray[3], V.E. Kimonis[1].* 1) Division of Genetics, Childrens Hospital, Harvard Medical School, 300 Longwood Avenue, Fegan 10, Boston, MA 02115; 2) Center for Medical Genetics, Molecular Genetics Laboratory, 1000 North Oak Avenue, Marshfield, WI, USA; 3) Provincial Medical Genetics Programme, Children's and Women's Health Centre of British Columbia C234, 500 Oak Street, Vancouver, British Columbia V6H 3N1; 4) Division of Bone and Mineral Diseases, Washington University School of Medicine, and Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO, USA.

Autosomal dominant progressive myopathy of a limb-girdle distribution and bone fragility is a rare and complex disorder of unknown etiology. In this report we present clinical studies and mapping of this disease utilizing a genome-wide scan. Affected individuals in the large family we describe demonstrate the phenotypic variability of this disease, developing combinations of progressive muscle weakness, susceptibility to fractures and poor healing of long bones. Additional features seen in the family include thin, prematurely grey hair, thin skin and hernias. Electromyograms and muscle biopsies show non-specific myopathic changes, and radiographs reveal coarse trabeculation, patchy sclerosis, cortical thickening, and narrowing of the medullary cavity. We identified linkage to chromosome 9p21-p22, with a maximal LOD score of 3.74 for marker DS91121. Haplotype analysis narrowed the disease locus to a 15Mb region flanked by markers AGAT142P and GATA5E06P. This new region contains the locus for Diaphyseal Medullary Stenosis with Malignant Fibrous Histiocytoma (DMS-MFH). Although DMS-MFH lacks the myopathy and causes malignant fibrous histiocytoma in 35% of affected individuals, the skeletal similarities to the family presented are striking. Identification of the gene for this unique disorder will facilitate our understanding of the complex interaction between bone and muscle pathogenesis in this and other related debilitating disorders.

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Bivariate linkage of cortical bone thickness and skeletal age to chromosome 3p. *D.L. Duren[1], J. Blangero[2], T. Dyer[2], S.A. Cole[2], R.M. Siervogel[1], B. Towne[1].* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX.

Skeletal growth, maturation, and mineralization are highly coordinated, yet distinct, processes of bone development. The genetic regulation of each process is also distinct, but at the same time it is likely that they have some shared genetic basis. In this study, we analyzed radiographic cortical thickness of the second metacarpal and skeletal maturation of the hand-wrist using the FELS method (Roche et al., 1988) in 600 children from the Fels Longitudinal Study examined at chronological age 10 years. These individuals are from 144 nuclear and extended families. An initial set of 440 of these individuals have been genotyped for some 400 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain uni- and bi-variate multipoint LOD scores. Univariate linkage analysis of cortical bone measures revealed three significant LOD scores (> 3.0) for linkage to markers on chromosomes 2 and 3. We followed this with a bivariate linkage analysis of lateral cortical thickness and skeletal age, and found significant linkage (LOD = 3.98) to markers on chromosome 3p at 32 cM (between markers D3S1304 and D3S1263), an increased linkage signal over that of lateral cortical thickness alone (LOD = 3.67) to the same chromosomal position. One potential positional candidate gene residing in this region is the oxytocin receptor gene (OXTR). Because the oxytocin receptor was recently found to be functional in both osteoblasts and osteoclasts, and has binding affinity to both oxytocin and estrogen, OXTR is a plausible candidate gene for childhood bone accrual. Future work will seek to identify other specific genes that influence the accrual of bone mass at different stages of childhood development. Supported by NIH grants HD36342, HD12252, and MH59490.

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A gene for Autosomal Dominant Childhood Onset Proximal Spinal Muscular Atrophy with Lower Limbs Predominance maps to chromosome 14q32.3. *L. Viollet[1], A. Toutain[2], L. Guyant-Marechal[3], J.M. Pedespan[4], C. Laroche[5], B. Fouquet[6], D. Lacombe[7], I. Maystadt[8], P. Saugier-Weber[3], A. Munnich[1].* 1) INSERM U-393, Hopital Necker, Paris, France; 2) Service de Génétique, Hôpital Bretonneau, Tours, France; 3) Service de Génétique, Hôpital Charles Nicolle, Rouen, France; 4) Service de Neuropédiatrie, Hôpital Pellegrin, Bordeaux, France; 5) Service de Neuropédiatrie, Hôpital Dupuytren, Limoges, France; 6) Service de Medecine Physique, Hôpital Bretonneau, Tours, France; 7) Service de Génétique, Hôpital Pellegrin, Bordeaux, France; 8) Centre de Génétique Humaine, UCL, Bruxelles, Belgique.

Spinal muscular atrophies (SMA) are characterized by progressive anterior horn cell degeneration, leading to motor weakness, muscular atrophy and denervation. Numerous SMA phenotypes have been reported, differing by the distribution of paralysis, the age at onset and the mode of inheritance. Here we report on a phenotype of autosomal dominant SMA in a large European pedigree, characterized by childhood onset and benign evolution. In all of patients, walking ability was delayed and weakness predominated at the pelvic girdle muscles. A symmetrical atrophy of the thigh and leg muscles was noted, contrasting with normal or slightly weak scapular girdle muscles. No chest deformity was noted, scoliosis was absent and patients were still ambulant at adulte age. Osteotendinous reflexes were preserved and/or brisk in the lower and upper limbs. Diagnosis of motor neuron disease was suspected on neurological assays, showing muscle denervation with normal motor and sensory nerve conduction velocities. SMN gene deletion was excluded in the DNA of probants. We performed genetic analysis in this pedigree by whole genome scanning and we identified a common haplotype in the 14q32.3 region located between loci D14S973 and D14S1007, cosegregating with the disease. (Zmax: 3,85 at ?=0.00), corresponding to a large physical distance of 13 megabases. Recruitment and genetic analysis of other cases of autosomal dominant SMA would be very helpful to narrow the critical region. Testing candidate genes in this region will probably allow identifying a new gene involved in motor neuron degeneration.

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Focal dystonia not linked to DYT6, DYT7 and DYT13 loci in two French families. *M.Y. Frederic[1], C. Dhaenens[2], C.M. Monino[1], S. Schraen[2], M. Martinez[3], M. Claustres[1], S. Tuffery-Giraud[1], B. Sablonniere[2], G. Collod-Beroud[1].* 1) Laboratoire de Genetique Moleculaire, IURC, Montpellier, France; 2) Laboratoire de Biochimie et de Biologie Moléculaire, CHU de Lille, Lille, France; 3) Inserm, EMI00-06, Evry, France.

Dystonia is a neurological movement disorder characterized by involuntary muscle contractions, which force certain parts of the body into abnormal, sometimes painful, movements or postures. Idiopathic torsion dystonia (ITD) includes adult onset dystonia which usually remains focal, affects one body part and occasionally spreads to adjacent regions. Commonly described forms of focal ITD (FITD) include cervical dystonia (spasmodic torticollis), blepharospasm, oromandibular dystonia, laryngeal dystonia (spasmodic dysphonia), and limb dystonia (among which task-specific dystonia such as the writers cramp). FITD are genetically heterogeneous. Three loci, DYT6, DYT7 and DYT13, have been already reported but no gene has been identified to date. We studied 2 large French families presenting with varied symptoms of FITD: spasmodic torticollis, postural tremor, blepharospasm, task-specific cramp and dysphonia. The first family, DYST143, is composed of 26 subjects (5 definitely affected, 2 with partial phenotype). The average of onset is 4320 years. The second family, DYST154, included 21 subjects (3 definitely affected, 2 with partial phenotype). The average of onset is 3216 years. DYST 143 and DYST154 were studied for the three loci known to be implicated in FITD by genotyping all individuals with markers spanning these regions. Haplotype construction showed no common haplotype shared among all definitely affected family members within the 3 regions. These results were confirmed by lod scores -2 for DYST 143. For DYST154, non informative lod scores for DYT 7 were obtained, but the implication of this locus would lead to a change of status for three subjects currently aged of more than 32 years old and scored as unaffected. These results illustrate the great genetic heterogeneity of FITD and indicate the existence of one or more unassigned genes for this pathology in the French population.

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Complete genomic screen for Parkinsons disease in Tunisia and evidence for the involvement of PARK8. *L. Ishihara[1], L. Warren[2], R. Gibson[2], S. Thomas[2], N. Hattori[3], A. Akkari[2], R. Elango[2], R. Amouri[4], M. Kefi[4], S. Belal[4], N. Gouider-Khouja[4], S. Ben Yahmed[4], A. Nelsen[2], C. Stapleton[2], D. Kelly[2], D. Leppert[2], L. Middleton[2], C. Brayne[1], F. Hentati[4].* 1) Public Health & Primary Care, University of Cambridge, United Kingdom; 2) Glaxo Smith Kline, RTP, NC and United Kingdom; 3) Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan; 4) Institut National de Neurologie, Tunis, Tunisia.

The etiology of Parkinsons disease (PD) is complex, with evidence suggesting that both genetic and environmental factors influence disease risk. Monogenic forms of the disease, including dardarin (PARK8), have been identified from familial linkage studies, and they have provided clues into the pathogenesis of PD and possible modes for therapeutic intervention. In an attempt to identify additional genetic causes of PD, a family-based genetic linkage study has been carried out as a collaboration between the pharmaceutical company GlaxoSmithKline and investigators from Tunisia. Eighty multiplex PD families were recruited from the Institut National de Neurologie in Tunis, which provides a specialized neurological service to the entire country. Due to the socio-cultural conditions and high rate of consanguineous marriage, the frequency of neurodegenerative disorders (including PD) is much higher in Tunisia than in other countries. These families provide a unique and powerful opportunity for performing genetic linkage studies. PD was diagnosed using UK PD Society Brain Bank Clinical diagnostic criteria, but postural instability was not considered to validate the diagnosis. Interrater confirmation of diagnosis was carried out, and clinical data and DNA collection methods were standardized. A whole genome scan was performed in these families with an average marker density of 4 centiMorgans. Preliminary analysis has shown a peak in the PARK8 region in a subset of families. One individual from each family will be sequenced for a mutation in PARK8 (G2019S), which has recently been reported to be much more frequent in North Africans than Caucasians. Linkage and sequencing results for PARK8 will be presented along with a detailed clinical review of relevant phenotypes.

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Genome-wide search for the *CLN7* gene. *E. Siintola[1], M. Topcu[2], A.-K. Anttonen[1], A.-E. Lehesjoki[1].* 1) Folkhälsan Institute of Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland; 2) Department of Pediatrics, Hacettepe University Faculty of Medicine, Section of Child Neurology, Turkey.

The neuronal ceroid lipofuscinoses (NCLs) are autosomal recessive neurodegenerative disorders characterized by the accumulation of autofluorescent storage material in many cells types, especially in neurons. At least seven subtypes of childhood-onset NCLs have been identified of which the late infantile onset forms (LINCLs) are genetically most heterogeneous with three underlying genes identified (*CLN2*, *CLN5*, and *CLN6*). A variant form of LINCL (vLINCL) present in Turkish patients has been considered a distinct clinical and genetic entity (CLN7). Clinical features in Turkish vLINCL patients include age of onset at 2-7 years, epileptic seizures, myoclonus, motor impairment, mental regression and loss of vision. On electron microscopy, condensed fingerprint profiles or a mixture of curvilinear and condensed fingerprint profiles are seen in many tissues.

We previously showed that in a subset of patients, Turkish vLINCL is caused by mutations in either the *CLN6* or the *CLN8* gene. In seven consanguineous families we excluded all known human NCL loci as well as three loci homologous for genes underlying NCL-like phenotypes in animal models (*CTSD*, *CLCN3*, and *CLCN7*), suggesting that these families represent "true" Turkish vLINCL. We undertook a genome-wide scan in these seven families using 378 microsatellite markers covering the human genome (modified Applied Biosystems LMS-2/MD10 marker set, Finnish Genome Center, University of Helsinki) and looked for regions with shared homozygosity between the families. No single genomic region showed overlapping homozygosity in all families. A region on chromosome 3 with overlapping homozygosity in six families was excluded after fine mapping in all but two families. We are currently analyzing regions with shared homozygosity between four or more families. These regions also show positive LOD scores in multipoint linkage analysis. Our data suggest the existence of more than one NCL-causing, novel genes in the Turkish patients.

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Genomewide-scan results for a large family affected with intracranial aneurysms. *D.J. Verlaan[1,2], A. Noreau[1], J. St-Onge[1], G.A. Rouleau[1].* 1) Faculté de Médecine, Centre Hospitalier de l'Université de Montréal-Hôpital Notre-Dame, Montréal, Québec, Canada; 2) Department of Human Genetics, McGill University, Montréal, Québec, Canada.

Background: Intracranial aneurysms (IA) are dilations of intracranial arteries that occur most commonly at arterial bifurcations. Unruptured IAs are present in approximately 1-2% of the population aged older than 30 years old. Aneurysms are only rarely symptomatic unless they rupture, which typically results in a subarachnoid haemorrhage that is associated with high morbidity and mortality. The purpose of our study is to map a gene that predisposes to IA.
Methods: A French Canadian family containing 8 affected individuals with IA was identified. Six affected cases and 5 unaffected individuals were sent for an 8 cM genomewide scan at deCODE (Reykjavik, Iceland). The disease segregation within the family was compatible with a Mendelian inheritance pattern, and a parametric LOD score approach was used to test for linkage. Multipoint LOD scores of the autosomes were calculated using GENEHUNTER version 2.1_r5 beta and two-point linkage for the X chromosome was calculated using MLINK from the FASTLINK 3.0P package. An affecteds-only approach was performed, using an autosomal dominant model, a phenocopy frequency of 0.01, a penetrance of 0.8, a disease allele frequency of 0.001 and deCODE allele frequencies.
Results: Preliminary multipoint analyses suggest five possible regions of linkage on chromosomes 4q22-34, 5q22-23, 8q21-22, 12p13 and 15q26. None of these regions have previously been implicated in the pathogenesis of IA, except for the 5q22-23 region, which as already been reported.
Conclusion: Further genotyping will permit us to determine the inclusion or exclusion of these positive regions. Genotyping of additional FC families may also help us determine where this susceptibility locus lies.

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X-linked lethal infantile spinal muscular atrophy (XL-SMA): Progress in disease gene identification. *M. Ahearn[1], J. Ramser[2], K.O. Yariz[1], D. Dressman[3], M. Barmada[4], E.P. Hoffman[3], M. Farrer[5], M. Hulihan[5], A. Meindl[2], L. Baumbach[1].* 1) Univ Miami Medical Sch, Miami, FL; 2) Ludwig Maximilians Univ, Munchen, Germany; 3) Children's National Med Ctr, Washington, DC; 4) Univ of Pittsburgh, Pittsburgh, PA; 5) Mayo Clinic, Jacksonville, FL.

X-linked lethal infantile motor neuron disease (MIM 301830), resembles Werdnig-Hoffman disease, with the additional features of early onset or congenital contractures and/or fractures. Fourteen unrelated families have been identified from North or Central America and Western Europe. Of these, nine families have been tested for X-chromosome linkage; all families mapped to the same Xp11.3-Xq11.2 region. One family is thought to represent a new mutation. The cumulative LOD score is 8.71 at a =0.0 at DXS1003. In the past year we have focused on the two largest families using more microsatellite repeat markers and SNPs. Markers were run on the ABI 3100 and allele calls made with GeneMapper. SNPs were selected from the ABI Assays-on-Demand and run on the ABI 7000. This has not yielded new recombination events however, new LOD score data has been generated which confirms the region of linkage. The highest LOD score region spans DXS8080 to DXS1055 with LOD score of 3.61-3.63 based on these two families. Analysis of these data and LOD score calculations has been independently verified by a second laboratory. Mutation screening through genes in the region has proceeded jointly between University of Miami (UM) and Ludwig Maximilians University (LMU) pursuing complimentary approaches. UM screening has focused on study of candidate disease genes based on presumed protein functions that relate to RNA processing, neuronal or muscle development, cell to cell communication and apoptosis. Growing knowledge concerning the molecular and cellular basis of autosomal SMA is used for selection many of these candidates. At LMU, all cDNAs in the disease gene region are being tested for mutations in XL-SMA samples using high-throughput methodologies. In total, approximately 80 genes within the candidate region have been tested with no mutations found in affected males or carrier females. Gene screening will be completed by October 2005.

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Mutational and linkage analysis in three families from Southern Italy with Autosomal Dominant Nocturnal Frontal Lobe Epilepsy. *E.V. De Marco[1], F. Annesi[1], S. Carrideo[1], P. Forabosco[2], I.C. Cirò Candiano[1], P. Tarantino[1], F.E. Rocca[1], D. Civitelli[1], P. Spadafora[1], A. Gambardella[3], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Mangone (Cosenza), Italy; 2) Institute of Population Genetics, National Research Council, Alghero (SS), Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

ADNFLE is caused by mutations in the 4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) gene. More recently we identified a second gene coding for the 2 subunit (CHRNB2)in a large Italian family. However, only a minority of ADNFLE families carry a mutation of either gene. Here, we investigated nine brain-expressed genes, encoding distinct neuronal nicotinic acetylcholine receptor (nAChR) subunits, in three additional ADNFLE families from Southern Italy. We first analysed DNAs from three probands, belonging to these families, that showed the typical ADNFLE phenotype. Exon 5 of CHRNA4 and CHRNB2 genes, harboring all the known mutations, were sequenced. Then, we performed a linkage study on 11 affected and 29 non affected individuals. Microsatellite markers encompassing the chromosome localization of the nAChR subunit genes and RFLPs were used for the linkage study. The following chromosome regions were examined: 1q21 (CHRNB2), 8p21 (CHRNA2), 8p11.2 (CHRNA6, CHRNB3), 15q14 (CHRNA7), 15q24 (CHRNA5/A3/B4), 20q13.2 (CHRNA4). Two point and multipoint linkage analyses were performed by LINKAGE and GENEHUNTER. No mutations in exon 5 of CHRNB2 or CHRNA4 were found. Moreover, linkage analysis excluded the involvement of these genes in ADNFLE in two families. The third family showed a not significant LOD score value for CHRNA4 markers. As regards the remaining loci, negative LOD scores values (less than -2) were obtained for all markers by parametric linkage analysis in one family, while the study is still in progress in the other two families. Our preliminary results further illustrate the considerable genetic heterogeneity for such a syndrom. It is reasonable to hypothesize that either new genes not belonging to nAChR gene family or yet unidentified brain-expressed nAChR genes are involved in the pathogenesis of ADNFLE.

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A new locus for temporal lobe epilepsy maps to chromosome Xp11-q21. *L. Patry[1], D.J. Verlaan[2,3], D.K. Nguyen[1], P. Major[1], A. Lortie[1], L. Carmant[1], I. Rouleau[1], G.A. Rouleau[2], P. Cossette[1].* 1) Service de Neurologie, Université de Montréal, Montréal, Québec, Canada; 2) Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada; 3) Department of Human Genetics, McGill University, Montréal, Québec, Canada.

Background: Thirty members of a French-Canadian family segregating temporal lobe epilepsy (TLE) were studied over 4 generations. All affected individuals (n=6) were male who developed stereotypical complex partial seizures between 2 and 14 years of age. All seizures were triggered in the course of showering or bathing (reflex epilepsy), which is also known as hot-water or bathing epilepsy.
Methods: The X chromosome was genotyped using 10 evenly distributed microsatellite markers because genealogical analysis of the family was compatible with a recessive X-linked pattern of transmission. Two-point parametric LOD scores were calculated using MLINK from the FASTLINK 3.0P package using a penetrance of 0.8, a disease allele frequency of 0.00001, a phenocopy frequency of 0.0001 and equal allele frequencies. Fine mapping was performed using 14 additional markers located within the linked region.
Results: Evidence for linkage was found on chromosome Xp11-q21. Fine mapping of the region revealed key recombinants at markers DXS8042 and DXS6799, delimiting the candidate region to a 27 cM interval. Linkage analysis of the region revealed a maximum LOD score of 3.08, at = 0, for markers DXS1003, DXS6949, DXS1125 and DXS8092.
Conclusion: We mapped a new locus for TLE on chromosome Xp11-q21. To our knowledge, this is the first locus identified for TLE on the X chromosome. Although X-linked inheritance has been rarely described for idiopathic epilepsy, a male predominance has been observed for some epileptic syndromes, including benign partial epilepsy of adolescence (3:1).

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Follow-up study of the 5q23 candidate region for Parkinsons Disease: Fine-mapping linkage results in the PROGENI-EU consortium data. *M. Martinez[1,2], N. Pankratz[3], A. Durr[4], B. Nichols[5], N. Wood[6], T. Faroud[3], PROGENI-EU consortium.* 1) INSERM EMI 00-06, Evry, France; 2) Northwestern University, Evanston, IL; 3) Indiana University School of Medicine, Indianapolis, IN; 4) INSERM,U289, Paris, France; 5) Cincinnati Childrens Hospital Research Center, Cincinnati, Ohio; 6) Institute of Neurology, London, UK.

The chromosome 5q23 region has provided evidence of linkage to PD susceptibility genes in 4 independent PD samples [Scott et al., 2001; Hicks et al., 2002; Prankratz et al., 2002; Martinez et al., 2004]. It has also been shown positive for linkage to age of onset to PD [Li et al., 2002]. The reported linkage peaks were, however, individually modests (lods=1.0-1.6), suggesting that the locus may have only modest effects, and/or may act in a subset of families. We have formed an International Parkinsons research group (PROGENI-EUGSPD), combining data from the 2 United States studies and several European centers. This leads to the largest family sample (528 families,~1 000 PD subjects and >420 Affected Sib Pairs) ever been made available for linkage analyses to PD. Here, we present results on the follow-up study of the 5q23 region (~79cM) using a common set of 20 microsatellite markers. Evidence for linkage to PD susceptibility was not significant whether a narrow or a broad definition of the disease was used. Linkage to AOO of PD was significant under the broad (lod=1.75, P=0.005) but not under the narrow (lod=0.51, P=0.13) phenotype. Altogether, the hypothesis of a 5q23 susceptibility PD gene is unlikely in the consortium data. Yet, genetic heterogeneity within our sample will be investigated. Our data supports the hypothesis of a 5q23 gene contributing to AOO variability. The results remain, however, puzzling. Inconsistent results can be explained by a decrease in power of the smaller sample of narrow PD cases, but also by a higher rate of false positive linkage finding resulting from some distributional properties of AOO under the broad model. The sensitivity of our linkage results to these factors will be evaluated using simulations to derive appropriate significance levels.

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Linkage and association study of Alzheimer disease families linked to 9p21.3. *C.R. Leon-Guerrero, S. Zuchner, P. Xu, C. Browning, P. Bronson, E. Martin, J.R. Gilbert, J.L. Haines, M.A. Pericak-Vance.* Duke University Medical Center, Center for Human Genetics, Durham, NC.

In a genome wide microsatellite-based linkage screen on Caucasian Alzheimer disease (AD) families we mapped a new chromosomal locus to 9p21.3. The peak lod score occurred in the subset of families with at least one autopsy confirmed AD case. This linkage was independently confirmed in AD families from a consanguineous Israeli-Arab community suggesting a recessive mechanism. Using an updated clinical sample of our 197 autopsy confirmed AD families, we genotyped 88 SNPs within the chromosomal region of approximately 20 Mbp. Analysis of multiple SNPs surrounding the original peak region generated further support for linkage with peak two-point lod scores of 2.59 at rs6475797 and 2.45 at rs6475797. These data potentially narrowed the region of interest to 2.7 Mbp with several AD candidate genes. Association analysis showed consistently significant results (p0.05 - 0.005) in the genes cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A, CDKN2B). Changes in expression of CDKN2A have repeatedly been reported in Alzheimer brains and a number of cyclin-dependent kinases are elevated in neurons prone to dedifferentiation and degeneration. We conclude that CDKN2A and CDKN2B are promising new candidate genes potentially contributing to AD susceptibility on chromosome 9p.

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Amphetamine-induced locomotion and microsatellite marker association in recombinant congenic strains of mice. *A. Torkamanzehi[1, 6], P. Boksa[1, 2], M. Ayoubi[1], N.M.K. Ng Ying Kin[1], E. Skamene[5], G. Rouleau[3, 4], R. Joober[1, 2].*
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Finding genes that influence complex disorders, such as schizophrenia, is one of the biggest challenges of present day human/medical genetics. The objective of this study was to map and identify, in a mouse model, microsatellite markers associated with amphetamine (AMPH)-induced locomotor activity, a rodent behavioral trait that models the hyperdopaminergic state postulated in schizophrenia. Two panels of recombinant congenic strains (RCSs) of mice derived from A/J (A) and C57BL/6J (B) were used in this experiment. The RCSs were informative for 620 microsatellite markers covering the whole genome. A total of 433 animals from 38 congenic and parental strains were tested. AMPH-induced total distance traveled (AMPH-TDIST) was recorded and used as the target phenotype for QTL analysis. Two strains were identified in the A/J background (A52 and A63) which significantly elevated AMPH-TDIST compared to their parental A/J strain. These strains behaved similarly to C57BL/6J, showing higher AMPH-TDIST, AMPH-TDIST peak values and AMPH-TDIST longer time to peak when compared to A/J. Markers on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 19 and 20 were significantly associated with AMPH-TDIST in the A background. Within the B background, four strains significantly deviated from the parental (B81, B74, B69 and B75). Two of these strains had significantly higher and two significantly lower AMPH-TDIST than the parental C57BL/6J. Markers associated with AMPH-TDIST in the strains with the B background mapped to chromosomes 5 and 20. Data from this approach combined with other genetic (mapping data in humans) and functional sources may help to identify candidate genes relevant to schizophrenia.

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Human substance abuse vulnerability loci: confirmation and fine mapping of rSA7 on chromosome 4q. *J.P. Gong, H. Ishiguro, G. Uhl.* Molecular Neurobiology Branch, NIDA, Baltimore, MD.

We have used pooled association genome scanning to identify differences in allelic frequencies at the SNP markers including rs1857 (WIAF-3821) between polysubstance abusers and controls in European-American (abuser/control allelic frequency ratio 0.85) and African-American (abuser/control ratio1.63) samples (Uhl et al, 2001). Sullivan et al (2005) have recently reported linkage to cocaine dependence to nearby markers. We now report confirmation of this association in individually-genotyped European- (minor allele frequencies 0.137 vs 0.175) and African-American (0.077 vs 0.019) samples. rs1857 lies between the 3 ends of annotated genes that encode the sprouty 1 antagonist of fibroblast growth factor signaling (SPRY1) and an apparent soluble guanylate cyclase (LOC441039). Alignments of BAC sequences and HapMap data from the central 95 kb segment of this region reveal nine haplotype blocks ranging from 2 - 24 kb. Dinucleotide repeat polymorphisms within three of these blocks of restricted haplotype diversity each display nominally-significant allelic associations with addiction vulnerability. SPRY1, LOC441039 and other interesting genes near these blocks of restricted haplotype diversity including FGF2 and NUDT6 are thus good candidates to contain allelic variants that influence vulnerability to substance abuse and contribute to the association signals in rSA7, at and near rs1857.

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Haplotype Analysis of the human Dopamine Receptor D4 (DRD4) Gene in the Japanese Schizophrenia. *H. Mitsuyasu, H. Kawasaki, L. Gotoh, Y. Kobayashi, N. Maeda, S. Kanba.* Dept Neuropsychiatry, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.

Schizophrenia is the most prevalent of the major psychotic disorders with 1% of the population affected worldwide. Although genetic factor is suggested to be involved in the etiology of schizophrenia, the underlying pathophysiologic mechanisms leading to the development of schizophrenia are still unclear. Several lines of evidence suggest the possible involvement of dopaminergic neurotransmission systems in the pathogenesis of schizophrenia. Thus,'gdopamine hypothesis'h emerges from the observation of altered dopamine levels in various regions of schizophrenic brains and the finding that some antipsychotic drugs block dopamine receptors. Dopamine receptors have been a focus of genetic studies with schizophrenia. In particular, DRD4, a member of the D2-like dopamine receptor family, has been suggested to be a strong candidate because of the finding of high affinity of clozapine for DRD4 and the elevation of DRD4 protein and mRNA expression postmortem in the brain of schizophrenia patients. In order to investigate the contribution of DRD4 dopamine neurotransmission systems to schizophrenia, we carried out a haplotype analysis of DRD4. We genotyped 216 schizophrenic patients (121 men and 95 women) and 243 healthy controls (138 men and 105 women) with 26 polymorphisms by direct sequencing method. Uni- and multivariable analysis showed significant gender-specific association between DRD4 polymorphisms and Japanese schizophrenia patients. Three SNPs (-809G/A, -713C/T, and -521T/C) are significantly different between female schizophrenia patients and controls. In contrast, -603del/T showed a significant difference only in male schizophrenia patients. Haplotype analysis using unphased data also showed significant differences. Our results indicate that the DRD4 gene might be involved in gender-specific pathophysiologic mechanisms that contribute to the development of schizophrenia. This study was approved by and performed in accordance with the guidelines of the Ethics Committee of the Graduate School of Medical Sciences, Kyushu University.

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Association analysis of adenosine A1, A2A, A2B, dopamine D1, and D2 receptor genes with schizophrenia in the Japanese population. *L. Gotoh, H. Mitsuyasu, H. Kawasaki, Y. Kobayashi, N. Maeda, S. Kanba.* Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan.

Antipsychotic agents used for the treatment of schizophrenia affect dopamine D2 receptor (DRD2) mediated neurotransmissions, suggesting that dopaminergic dysfunction plays an important role in the pathophysiology of schizophrenia. It is known that SCH23390, a se-lective dopamine D1 receptor (DRD1) antagonist, inhibited PCP-induced schizophre-nia-like behaviors. It is also reported that adenosine neurotransmitter system has functional interaction with dopaminergic neurotransmission. Therefore, it is possible to hypothesize that the functions of adenosine A1, A2A and A2B receptors (ADORA1, ADORA2A and ADORA2B) could be some part of the pathophysiological mechanisms of schizophrenia. To clarify the relationship between five receptors and schizophrenia, the polymorphisms (SNPs) of these five receptor genes were analyzed in both schizophrenia patients and nor-mal controls. Total 30 fragments for genotyping experiments were amplified by PCR from each subject consisting of the schizophrenia patients (n=100) and normal controls (n=100). Primers were designed based on the positions of SNPs based on the database of JSNP (Japanese Single Nucleotide Polymorphisms). Using the amplified DNA fragements, all the subjects were genotyped with 27 SNPs and 2 deletions in ADORA1 gene, 4 SNPs in ADORA2A gene, 1 SNP in ADORA2B gene, 1 SNP in DRD1 gene, and 18 SNPs in DRD2 gene. Based on the results, genotyping and allele frequencies were calculated. Association analysis of each polymorphism was performed between schizophrenia patients and normal individuals. So far, no significant difference was identified. Haplotype prediction, linkage disequilibrium calculation, and multi-variate statistical analysis are carried out. All subjects were given informed consent before blood collection strictly based on the ethical regulations of Kyushu University.

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Association analysis of human cAMP-GEFII gene polymorphisms with Japanese schizophrenia patients. *H. Kawasaki, H. Mitsuyasu, Y. Kobayashi, L. Gotoh, N. Maeda, S. Kanba.* Dept Neuropsychiatry, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.

We have previously reported novel genes of second-messenger regulated Rap1-GEF (guanine nucleotide exchange factor) gene family whose GEF activities are positively regulated by the binding of second-messenger molecules such as cAMP, calcium and diacylglycerol (DAG), indicating that three major second messengers transduce their signals to target molecules different from protein kinases. A part of this gene family includes cAMP-GEFI and cAMP-GEFII, CalDAG-GEFI and CalDAG-GEFII. Both cAMP-GEFI and cAMP-GEFII have binding domains for cAMP as well as GEF domains. Since antipsychotics have antagonistic actions for dopamine receptors regulating intracellular cAMP concentrations in central nervous system, cAMP-GEFs can be good candidates for molecular studies of schizophrenia. In order to investigate the contribution of cAMP-GEFII to the pathophysiological mechanisms of schizophrenia, we carried out a genetic analysis of single nucleotide polymorphisms (SNPs) of the cAMP-GEFII gene. Information of a total 21 SNPs was collected based on the databases of both dbSNP and JSNP and our genotyping experiments, which included 3 coding SNPs and 17 intron SNPs and one regulatory SNP. We found two novel non-synonymous ones. We genotyped 96 schizophrenic patients and 112 healthy controls with the 21 SNPs by direct sequencing method. The results of haplotype prediction, linkage disequilibrium calculation, and multi-variate statistical analysis are now being analyzed. Although there was slightly different distribution of the regulatory SNP between schizophrenia and controls, no statistical significant association were found. All subjects were given informed consent based on the ethical regulations of Kyushu University.

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An oligogenic segregation and linkage analysis of non-P53 Li-Fraumeni syndrome families. *E.W. Daw, C.C. Wu, S.E. Olufemi, J. Ma, L.L. Bachinski, C.I. Amos, R. Krahe, L.C. Strong.* Univ Texas MD Anderson CA Ctr, Houston, TX.

Li-Fraumeni syndrome is a rare inherited disorder that increases the risk of developing certain types of cancer, especially in the childhood to early adult years. The spectrum of cancers include soft tissue sarcoma, breast cancer, osteosarcoma, breast cancer, and others. Genetic mutations have been identified that cause this disorder, including mutations in the P53 and Chek2 loci. In four families we have identified, causative mutations have not yet been identified in either of these loci nor is there evidence for linkage to them. We undertook an oligogenic simultaneous segregation and linkage analysis of these families to identify other genes that might cause Li-Fraumeni syndrome. Our analysis used Bayesian Monte Carlo Markov chain methods (implemented in Loki) to simultaneously estimate the number, location and effect of genes that contribute to cancer liability, as measured through age-at-onset. There were 358 individuals included in the 4 families we studied, with 168 men and 190 women. The families ranged in size from 65 to 112. Phenotype and covariate data were available on 340 individuals, including 87 cancer-affected and 253 unaffected individuals. We used age-at-onset as a censored trait so that both affected and unaffected individuals contributed information to our analysis. The covariates included were sex and birth-year cohort (assigned categorically in 20-year intervals). Initially, we screened 373 markers across the genome in 57 individuals. These were followed up by 67 additional markers in regions of interest, typed on a slightly larger group of individuals. In the segregation part of our analysis, we found a > 99% posterior probability that at least one gene contributing to Li-Fraumeni syndrome was segregating in these families, and a >75% posterior probability that two or more genes were segregating. Both sex and birth-year effects were found, with the younger cohorts found to be at greater risk. Two chromosomes were identified as having a high posterior probability of linkage: Chromosome 6q25 (Bayesian L-score of 32.0) and chromosome 1q31 (Bayesian L-score of 35.2).

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High density mapping studies on chromosomal regions 1, 3, 6, 12, 13 and 17 in 28 families with chronic lymphocytic leukemia. *D. Ng[1], G.E. Marti[2], L. Fontaine[3], J.R. Toro[1], N. Caporaso[1], L.R. Goldin[1].* 1) Genetic Epidemiology Branch, DCEG, NCI, National Institutes of Health, DHHS, Bethesda, MD, USA; 2) Laboratory of Medical and Molecular Genetics, Division of Cell Gene Therapy, Food and Drug Administration, Bethesda, MD, USA; 3) Westat Research Inc., Rockville, MD, USA.

B-cell chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults living in western countries. There is accumulating evidence that a subset of CLL is due to genetic susceptibility. Population and family studies have shown an increased risk of lymphoproliferative disease among relatives of patients with CLL. To date, a causative factor has not been identified for familial CLL. Since 1974, the Genetic Epidemiology Branch (Division of Cancer Epidemiology and Genetics, National Institutes of Health) has enrolled families with two or more living cases of CLL. In 2003, we reported results of a genome wide scan of 18 CLL families. Lod scores of 1.0 or greater were detected on chromosomes 1, 3, 6, 12, 13 and 17. In an ongoing effort to identify genetic susceptibility loci for familial CLL, we performed a fine mapping study of chromosomal regions identified by the whole genome scan with 28 CLL families. Results from the fine mapping study showed no evidence of linkage at 1p22.1-p21.2, 3q22.1, 3q26.2, 6q22.31-q23.2, 12q24.23, 14q32.13, 17p13.3. Chromosome 13q22.1 remains a region of interest with a p-value of 0.014 and warrants additional molecular investigation as a susceptibility locus for CLL.

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A genome-wide linkage scan for left ventricular outflow tract malformation susceptibility loci. *K.L. McBride[1], S.D. Fernbach[2], G.A. Zender[1], S.M. Leal[2], D.W. Stockton[2], J.A. Towbin[3], J.W. Belmont[2].* 1) Center Molecular & Human Gen, Columbus Child Research Inst, Columbus, OH; 2) Dept of Mol & Human Genetics, Baylor College of Medicine, Houston TX; 3) Pediatric Cardiology, Baylor College of Medicine, Houston TX.

Malformations of the left ventricular outflow tract (LVOT) are relatively common (birth prevalence of 1/1000 live births) and carry high mortality. LVOT malformations include congenital aortic valve stenosis (AVS), coarctation of the aorta (CoA), and hypoplastic left heart syndrome (HLHS), which are related developmentally by obstruction of blood flow through the left side of the heart. Previous inheritance analysis of these malformations demonstrated both high heritability and sibling recurrence risks. Complex segregation analysis was compatible with an autosomal dominant inheritance in some families and an overall pattern of inheritance involving from 1 - 6 genes. These findings suggested linkage analysis may be successful in mapping susceptibility genes. 25 families comprising multiple members affected with AVS, CoA or HLHS were recruited. A total of 132 individuals were genotyped at 380 autosomal microsatellite markers spaced an average of 10 cM over the genome using the ABI Prism Linkage Mapping Set V2.5. Parametric linkage analysis under an autosomal dominant model was executed under FASTLINK, with additional analysis assuming heterogeneity performed with HOMOG. Regions with lod scores > 1.0 were considered suggestive of linkage. The highest lod scores were found on chromosomes 2p25.1 (HLOD = 1.95), 15q13.3 (1.44), 13q22.1 (1.17), and 10q21.2 (1.12). The region around 2p25.1 contained an interesting candidate gene, SMYD1. Knock out of this transcription factor gene in the mouse is characterized by left ventricular hypoplasia. Sequencing the coding region of SMYD1 in this cohort, however, did not uncover any segregating causative mutations. This study has identified several genomic regions where further research may reveal susceptibility loci for LVOT malformations. To this end, an additional 10 families (total 192 individuals in study) have been recruited for genotyping.

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Neuropeptide Y gene variants are linked to and associated with premature coronary artery disease in two independent datasets. *S.H. Shah[1,2], L. Wang[2], J. Rose[2], D.C. Crossman[3], C.B. Granger[1], J.L. Haines[4], C.J. Jones[5], V. Mooser[6], C. Haynes[2], B. Pedersen[2], P. Goldschmidt-Clermont[1], J. Vance[2], E.R. Hauser[2], W.E. Kraus[1].* 1) Duke University Department of Medicine, Durham NC; 2) Duke University Center for Human Genetics, Durham NC; 3) University of Sheffield School of Medicine, Sheffield, United Kingdom; 4) Vanderbilt University School of Medicine, Nashville TN; 5) University of Guelff, Cardiff, United Kingdom; 6) GlaxoSmithKline, Inc., Philadelphia PA.

Neuropeptide Y (NPY) has cardiovascular activity. NPY gene variants have been associated with obesity and vascular disease. We sought to investigate NPY variants in sporadic and familial premature coronary artery disease (CAD), based upon its location within a linkage peak in the GENECARD study of premature CAD. Linkage analysis was performed in 420 GENECARD families with microsatellite markers. Ordered subset analysis (OSA) using age-of-onset and lipids was done to reduce heterogeneity. Four NPY single-nucleotide polymorphisms (SNPs) (1 nonsynonymous (rs16139); 2 synonymous (rs5572, rs5574); 1 non-coding (rs5573)) were tested for linkage and association. To validate, an association study was done in an independent dataset of sporadic premature CAD (461 cases, 310 controls). We had previously shown linkage to chromosome 7p15 in GENECARD (multipoint LOD 1.04). OSA using microsatellites revealed a multipoint LOD of 4.22 in this region (p=0.004 for LOD increase) in 97 families with earliest age-of-onset (mean 37.8 years), who also had higher cholesterol. Two NPY SNPs were linked to premature CAD (rs5573 and rs5574, two-point LODs 1.87 and 2.18). Pedigree-disequilibrium testing showed these SNPs were also associated with CAD in GENECARD (p=0.04, 0.01). In the independent dataset of sporadic CAD, rs5573 and rs5574 were also associated with premature CAD (allelic OR 1.3 (p=0.03) and 1.3 (p=0.01); genotype OR 1.5 (p=0.02) and 1.6 (p=0.007)). Therefore, NPY variants appear to be linked to and associated with premature CAD. The nonsynonymous SNP was not associated; therefore, these SNPs may play a regulatory role. Evaluation of other SNPs and functional assessment are ongoing.

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Genetic loci on chromosome 3q are associated with variations in HDL cholesterol levels in a study of coronary artery disease. *E.R. Hauser[1], S.H. Shah[1,2], L. Wang[1], C. Nelson[3], P. Goldschmidt-Clermont[2], J. Vance[1], W.E. Kraus[2].*
1) Duke University Center for Human Genetics, Durham NC; 2) Duke University Department of Medicine, Durham NC; 3) Duke Clinical Research Institute, Durham NC.

Coronary artery disease (CAD) and dyslipidemia have genetic components. We have shown linkage to premature CAD on chromosome 3q13 (multipoint LOD=3.5), also shown to map to HDL cholesterol (HDLC). We then performed association fine-mapping in an independent dataset with two case groups (old (OA) and young (YA) CAD subjects) and a control group (ON). This revealed association of multiple single-nucleotide polymorphisms (SNPs), primarily in OA. The greatest clinical difference between the groups was in HDLC. We therefore sought to evaluate relationships between HDLC and SNPs in this region. Seventy-seven SNPs spanning 2 megabases (Mb) were genotyped in 169 OA, 301 YA, and 205 ON. Measured genotype analysis was performed using mean HDLC by genotype separately for each group, using linear models adjusted for sex and race. We found that HDLC was lower in YA (mean 38.9 mg/dL, SD 11.5) than OA (mean 44.3 mg/dL, SD 15.2), and lower in OA than ON (mean 49.3 mg/dL, SD 17.4). Significant measured genotype results were found primarily in OA. Seven SNPs showed significant (p0.05) differences in HDLC by genotype in OA: RS6788787 (mean HDLC(SD) by genotype (11, 12, 22, respectively): 15.0(14.1), 44.1(17.7), 41.3(13.1), p=0.02); RS6438389 (24.1(11.7), 45.6(15.3), 41.9(13.9), p=0.01); RS2927275 (40.7(20.7), 45.1(15.2), 37.5(13.4), p=0.05); RS1501881 (55.0(17.8), 42.2(15.5), 36.2(10.7), p=0.007); RS1910040 (53.0(31.6), 43.3(14.0), 38.2(14.6), p=0.04); P0356 (89.0(n=1), 42.7(12.2), 40.9(15.2), p=0.0002); and RS9861188 (52.7(18.6), 36.6(13.3), 43.7(13.6), p=0.01). Seven more SNPs were borderline significant (p<0.10). Trends were consistent in YA and ON. These SNPs clustered within 0.4 Mb in multiple linkage disequilibrium blocks. We conclude that variants in a 2 Mb region of chromosome 3q13, residing in a linkage peak for premature CAD, are associated with HDLC in sporadic CAD. We are evaluating whether this is a gene-environment interaction, a QTL for a primary CAD risk factor, or pleiotrophy.

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Searching for a gene on chromosome 11q influencing age-related blood pressure variation in Mexican Americans. *S. Rutherford, G. Cai, J.W. MacCluer, J. Blangero, L.D. Atwood, A.G. Comuzzie, S.A. Cole.* Dept Genetics, SW Foundation Biomed Research, San Antonio, TX.

Hypertension is a common disease and a well-known predisposing risk factor for stroke, renal failure and cardiovascular disease (CVD). Unraveling the genetic basis of common diseases of complex inheritance and unknown etiology such as hypertension has been complicated by genetic heterogeneity, incomplete penetrance, and the contributions from multiple environmental factors and their interactions. Despite these obstacles, we detected strong evidence for linkage (LOD score = 5.4) to age-related blood pressure (BP) traits near the marker D11S4464 on chromosome 11q24.1 in Mexican Americans from the San Antonio Family Heart Study (SAFHS). We are currently extending our initial linkage results by investigating single nucleotide polymorphisms (SNPs) within known genes located in the 1-LOD score interval surrounding the linkage peak near the D11S4464 marker and conducting association analyses to identify the most promising candidate genes. To date, we have genotyped 30 SNPs in 25 genes, of which 19 are reasonable BP candidates, within an 8.7 Mb region. Although additional SNPs within known genes are currently being genotyped to more thoroughly investigate this region, preliminary analysis of the current 30 SNPs shows association with age-related BP variation for a SNP located in the intron for the ubiquitination factor E4A gene (UBE4A; P value = 0.012) and another located in the intron for the Rho guanine nucleotide exchange factor 12 gene (ARHGEF12; P value = 0.016). Although a search of the literature could not produce previous studies implicating UBE4A to BP variation, a study with the stroke prone spontaneously hypertensive rat indicates that the Rho guanine nucleotide exchange factors may contribute to increased activation of the RhoA/Rho-kinase pathway, which regulates vascular tone and is implicated in hypertension. The SNP association result obtained in our Mexican American family provides additional motivation for our continuing search to identify an age-related BP variation gene on chromosome 11q.

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COMPLEX DISEASES IN ISOLATED POPULATION OF CILENTO: STUDY OF HYPERTENSION IN CAMPORA. *M. Ciullo[1], C. Bourgain[2], T. Nutile[1], C. Bellenguez[2], V. Colonna[1], M. Astore[1], A. Calabria[1], O. Guardiola[1], G.L. Iovino[3], B. Trimarco[3], M.G. Persico[1].* 1) IGB-ABT, CNR, Naples, Italy; 2) INSERM U535, Villejuif, France; 3) Department of Internal Medicine, Federico II University of Naples, Italy.

Essential hypertension (EH), is a major predisposing factor to renal failure and cardiovascular diseases. To identify genes involved in EH, we studied the population of Campora, an isolated village from Cilento, South Italy, with a few founders and inbreeding. The clinical assessment of a 443 random individual sample revealed that 39% were affected. All 443 individuals that related through a 2947-member pedigree spanning 15 generations, were genotyped for 1122 microsatellites on the genome (average marker distance: 3.6 cM). To perform non parametric linkage analysis, the pedigree was broken into 37 families with the maximum partitioning approach of Falchi et al (2005). A strong evidence for linkage (max Zlr score = 3.66) was found on chromosome 8q22-23. To protect from possible spurious linkage signals due to the pedigree breaking, significance was assessed with simulations taking this pedigree breaking step into account (genome-wide P-value = 0.08). Finally, the robustness of the signal to the breaking method is validated using a new method based on factor analysis. These results suggest that we identified a new hypertension-susceptibility locus on chromosome 8q22-23, a region with interesting candidate genes involved in vascular remodeling and angiogenesis.

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Auriculo-condylar syndrome: confirmation of wide intrafamilial clinical variability and mapping of the disease gene. *M.R. Passos-Bueno[1], F. Poerner[2], C. Masotti[1], K. Oliveira[1], R.M. Zechi-Ceide[3], M.L. Guion-Almeida[3], A. Splendore[1], R.S. Freitas[2].* 1) Dept Genetics and Evol Biol, Univ De Sao Paulo, Sao Paulo SP, Brazil; 2) CAIF, Centre for Integral Care to Cleft Lip and Palate patient,Curitiba, PR, Brazil; 3) Hospital de Reabilitação de Anomalias Craniofaciais, São Paulo, SP, Brazil.

Auriculo-condylar syndrome (ACS) is an autosomal dominant condition, characterized by malformed years (mostly characterized as question mark ears), micrognathia, glossoptosis, microstomia, condylar agenesis and cleft palate. To date there are very few reports of the syndrome in the literature, but ACS is clearly a branchial arch disorder, which is a group of disorders still very poorly characterized. The identification of the ACS gene will certainly contribute for a better understanding of the molecular events that take part in the development of the first and second branchial arches. In this report, we will describe an additional large Brazilian family, which includes 10 affected individuals with vertical transmission of the disease in 4 generations. We also noticed a wide spectrum of clinical variability, where the mildest cases present only the question mark ears while the most severe ones present the full phenotype. It is also our aim to map and identify the ACS gene. For this purpose, we have obtained DNA samples of affected and healthy individuals of this family as well as from another ACS Brazilian family, which clinical data was previously reported (Guion-Almeida ML et al, Am J Med Genet., 2002 112(2):209-14). We have first performed segregation analysis for chromosomal regions that contain genes associated with diseases of the first and 2nd branchial archs: 5q31-33, contains TCOF1, the gene for Treacher Collins syndrome; 16q12.1, includes the gene SALL1, responsible for Townes-Brocks syndrome; 14q32, candidate region to hemifacial microsomia. No evidence of linkage was observed with any of the markers tested in these regions, therefore, we excluded SALL1, TCOF1 and the 14q32 region as responsible for ACS. We are currently doing a genome scan, and 7 chromosomes have been excluded. FAPESP, CNPq, CEPID.

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QTLS controlling morphology in the domestic dog. *K. Chase[1], N.B. Sutter[2], D.R. Carrier[1], F.R. Adler[1], H.G. Parker[2], V. Oza[2], E.A. Ostrander[2], K.G. Lark[1].* 1) Department of Biology, University of Utah, Salt Lake City, UT; 2) Naional Human Genome Reserach Institute, NIH, Building 50 Bethesda MD 20892.

An early promise of the dog genome project was that we would identify genes important in morphology, as are reflected in the enormous variation in size, body proportion, and skull shape observed between established breeds of domestic dog. Towards this end we have used principal component analysis of skeletal variation in a population of Portuguese Water Dogs (PWDs) to reveal systems of traits defining skeletal structures. Morphological variation segregating within this breed, the breeds recent creation from few founders, and the availability of excellent pedigree records make possible a quantitative genetic analysis. Skeletal measurements from radiographs and a genome wide microsatellite scan were collected on 463 dogs. Based on 42 metrics from the pelvis, fore- and hind-limbs we have estimated size as the first principal component of skeletal variation. A total of 21 percent of variation in skeletal size of PWDs results from differences between females (smaller) and males. More than half of this sexual dimorphism results from an interaction between a QTL associated with CHM on the X-chromosome and a QTL associated with FH2017, linked to IGF1 on CFA15. In females, the small size associated CFA15 haplotype is dominant. In males the large size associated haplotype is dominant. These data suggest a genetic mechanism for the evolution of size sexual dimorphism in other species consistent, with the 1950 postulate termed Rensch's Rule. Positional cloning of the underlying genes and identification of relevant variants is underway using SNP-based haplotype approaches. Thus far, SNP haplotypes associated with large and small PWD have been established and studies are currently being extended to additional breeds.

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Linkage Analysis with Markers in Linkage Disequilibrium. *R. Pruim[1,2], M. Boehnke[1], G. Abecasis[1].* 1) Center for Statistical Genetics, Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI; 2) Department of Mathematics and Statistics, Calvin College, Grand Rapids, MI.University of Michigan, Ann Arbor, MI.

Single-nucleotide polymorphisms (SNPs) are rapidly replacing microsatellites as the marker of choice for genetic linkage studies. With the advent of ever denser SNP panels, it is inevitable that datasets will include markers that are in linkage disequilibrium. The standard analyses of these data, however, are based on an assumption of linkage equilibrium, and a violation of this assumption has been shown to distort linkage evidence in certain study designs. Recent work of Abecasis and Wigginton describes an efficient approach for modeling linkage disequilibrium between markers during multipoint analysis of human pedigrees. This method allows for rapid estimation of allele and haplotype frequencies within clusters of tightly linked markers but requires that the markers be partitioned into such clusters, and that the clusters have modest size (generally at most ~20 markers).

Here we describe a flexible, efficient algorithm for partitioning markers into clusters suitable for this kind of analysis. Our algorithm takes a greedy approach and attempts to maintain as much information for linkage as possible, even when some markers must be removed to achieve acceptable cluster sizes. Our algorithm estimates information loss based on marker counts, heterozygosity, or entropy measures. As an illustration of the method and of the dangers of ignoring linkage disequilibrium, we examine real and simulated data from dense sets of SNPs and microsatellites on chromosome 14.

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MCMC-based multipoint linkage analysis for two loci plus a polygenic component and general pedigrees. *Y. Sung, E. Thompson, E. Wijsman.* University of Washington, Seattle, WA.

Complex traits are influenced by multiple genes. Linkage analysis can be carried out with model-free approaches, but these have lower power for linkage detection than model-based approaches. Most programs for parametric linkage analysis (lod score analysis) allow only a single gene. PAP allows one gene plus a polygenic component, and TMLINK allows two genes for discrete traits. Modeling two linked genes as one can lead to incorrect inference about location; two-locus models have higher power for linkage detection and provide more accurate localization. However, two-locus analyses are computationally challenging and practical issues limit the number of markers and pedigree sizes.

We describe a new MORGAN program, lm_twoqtl, for parametric linkage analysis of a quantitative trait model having one or two linked genes and a polygenic component. We compute the likelihood with MCMC realization of segregation indicators at trait loci conditional on marker data, summation over founder genes at trait loci, and peeling of the polygenic component. Adding a polygenic component models additional genetic variability from other genes. This is the first two-locus lod score program that allows a polygenic component, and allows any number of markers and general pedigrees. It has been verified against exact computation, using PAP and an independent implementation in R.

Two simulated examples, both consisting of 100 nuclear families, show that two-locus analysis correctly identifies the locations of two linked genes with one large peak lod score (Z) near the true bivariate position and no misleading peaks. In the first example, where two genes contribute equally to the trait, one-locus analysis gave Z = 4.6 and Z = 5.9 near the true loci and a misleading peak with Z = 4.6 midway between them, whereas two-locus analysis gave a correct bivariate peak with Z = 19.8. In the second example, where two genes contribute unequally to the trait, one-locus analysis gave Z = 8.7 and Z = -1.3 near the true loci, thus missing the latter locus, whereas two-locus analysis gave a correct peak with Z = 22.5.

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Comparison of the information content of 100,000 SNPs, haplotype blocks and tag SNPs. *C. Rosenow.* Systems Biology, Agilent, Santa Clara, CA.

Agilent has developed a novel software tool for the analysis of genotyping data. GeneSpring GT 2 has been tested using more than 100,000 SNPs and 500 samples for linkage (> 21 bits) and association applications. It contains an integrated pedigree viewer with de novo pedigree building capabilities, solely based on raw genotype information, and can deduce haplotypes from genotype information. For family based applications we support parametric and non-parametric linkage analysis and transmission disequilibrium test (TdT). For unrelated individuals we provide quantitative and qualitative case control analysis, ANOVA and multivariate regression algorithms. We used the published genotype data from the HapMap project in GeneSpring GT 2 to deduce haplotype blocks and identified tag SNPs which are representative for each block. A case control analysis was performed on an unrelated Caucasian data set with i) the tag SNPs; ii) the deduced haplotype blocks and iii) the raw genotype data. The results of this comparison will be presented.

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EM2: Enhanced Computational Algorithm for Haplotype-Based Association Analysis in Case-Control Studies. *I. Amundson, K. Bradley, B. Yaspan, J.R. Smith.* Vanderbilt University Medical Center, Departments of Medicine and Cancer Biology, Nashville, TN.

Haplotype-based association analysis within a case-control study design is broadly anticipated to offer a powerful approach to identify genetic variants causing common disease. Accurate assignment of phase to enable tests of heterogeneity between case and control haplotype profiles is computationally very demanding. Use of permutation testing to estimate exact P values compounds this demand. This can limit the feasibility of large studies. We describe modification of algorithms implemented in the sliding window program of Fallin and Schork (AJHG 67:947-959, 2000) to facilitate large-scale studies. This enhanced version employs parallel-processing, enables use of multi-allelic as well as bi-allelic markers, and places no intrinsic limit on the number of markers or samples. Improved computational algorithms will be crucial for large-scale genome-wide association studies using a case-control design.

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Ordered subset analysis: new software and reporting guidelines. *B. Browning.* Genetic Data Sciences, GlaxoSmithKline, Research Triangle Park, NC.

Ordered Subset Analysis (OSA) [Hauser et al 2004] identifies subsets of families ordered by a trait-related covariate that give maximum evidence of linkage. Families are rank-ordered by a trait-related covariate, the ordered subset of families yielding the strongest evidence of linkage is identified, and the p-value is estimated using a permutation test.

The FLOSS (Flexible Ordered Subset) software package provides a new, flexible framework for OSA. FLOSS can be used with any linkage software package that reports NPL z-scores for each locus and family. In addition, linear ASM LOD scores can also be used in place of z-scores when using MERLIN. P-values are estimated using the efficient Besag-Clifford permutation test [1991]. FLOSS also includes a flexible program for calculating family covariate scores. FLOSS is written in Java for easy portability, and includes user documentation. The source code is documented, extensible, and freely available from the author.

Reporting Guidelines: We propose the following analysis and reporting guidelines:

1) Report the rationale for the specific covariates used in the analysis.

2) Report statistics describing the number of individuals with covariate data per family that were used to assign the family covariate scores.

3) Determine a significance threshold prior to analysis, and report all significant Monte Carlo p-values. The Monte Carlo p-values, not the increase in LOD score, should be emphasized.

4) Report the total number of regions tested using OSA and the covariates used in each region.

5) When two or more covariates yield significant p-values in the same region, give an appropriate measure of the correlation of the family covariate values.

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Genome scans for nonsyndromic cleft lip with or without cleft palate in families from seven countries. *M.L. Marazita[1], J.C. Murray[2], A. Lidral[2], M.E. Cooper[1], T. Goldstein McHenry[1], B.S. Maher[1], L. Moreno[2], S. Daak-Hirsch[2], L.L. Field[3], M. Arcos-Burgos[4], C. Valencia[4], J. Risk[5], J.T. Hecht[6], K. Doheny[7], E. Pugh[7], C. Boehm[7].* 1) Ctr for Craniofacial and Dental Genet, U of Pittsburgh; 2) U of Iowa; 3) U of British Columbia, Canada; 4) U of Antioquia, Colombia; 5) U of Liverpool, U.K; 6) U of Texas, Houston; 7) CIDR, Johns Hopkins U.

In recent years there has been substantial progress in identifying genetic factors that contribute to nonsyndromic cleft lip with or without cleft palate (NSCL/P). A combination of the evaluation of candidate genes and regions with genome-wide scans has proven fruitful. To identify genetic regions likely to include NSCL/P susceptibility loci, we performed genome-wide scans of 6,565 individuals (4,373 genotyped of whom 1,514 were affected) from 820 families ascertained from seven countries (Philippines, Colombia, China, India, Turkey, U.S.A., U.K.). Genotyping of the 10 cM genome-scan microsatellite markers in all study participants was performed by the Center for Inherited Disease Research (CIDR). The data was analyzed by a combination of linkage approaches: two-point and multipoint, parametric (LOD, HLOD under both dominant and recessive models) and non-parametric (NPL). A Bayesian approach utilizing the posterior probability of linkage (PPL) was also applied (see Govil et al, this meeting). A number of regions had genome-wide significant HLOD results (i.e. multipoint HLOD > 3.2): chromosome 1q22.3-41 (max multipoint HLOD=3.3, dominant model), 2p13 (3.2, dom), 3q26-q28 (4.1, dom), 9q22-q33 (5.4, dom), 14q24-q31 (4.2, rec), 16 (3.6, rec). The multipoint NPL results supported regions on chromosomes 1, 2, 6, 9, 12, 14 with p-values < 0.05 for each. The two-point PPL approach supported the chromosome 1, 2, 6, 9 and 12 regions (Govil et al). Follow-up studies including fine-mapping and candidate gene analysis of each of these positive regions is underway (see Mishima et al, Hart et al, this meeting). NIH grants DE09886, DE12472, DE08559, DE13076, DE14667, DE016148, DE016215, RR-00084; CIDR NIH contract N01-HG-65403.

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Coincident linkage of type 2 diabetes, metabolic syndrome, and cardiovascular disease in the Diabetes Heart Study. *C.D. Langefeld, J. Ziegler, J. Carr, B. Freedman, S. Rich, L. Wagenknecht, D.W. Bowden.* Wake Forest Univ. School of Medicine, Winston-Salem, NC.

Cardiovascular disease (CVD) is a major contributor to morbidity and mortality in type 2 diabetes (T2DM). The Diabetes Heart Study is a single center study of >450 T2DM-enriched families extensively phenotyped for measures of CVD, including calcified coronary plaque (CCP), in addition to T2DM, and metabolic syndrome (MS). 982 Caucasian subjects from 358 pedigrees (575 T2DM relative pairs) with at least two individuals with T2DM were included in a microsatellite-based a 10cM genome scan performed at the Mammalian Genotyping Center. Relationships and Mendelian errors were examined using the entire dataset with PREST and PEDCHECK. Sample meanSD age and BMI was 619 and 32.67.5, respectively. The sample was 53% female and 80% of the subjects have a diagnosis of T2DM and 80% have detectable CCP. Prevalent CVD was measured using 2 definitions: CVD1 based on self-reported history of CVD (463 subjects) and CVD2 defined as CVD1 and/or CCP greater than 600 (696 subjects). Additional qualitative traits evaluated in this analysis are +/- presence CCP and +/- carotid calcified plaque (CarCP). Multipoint nonparametric linkage analysis using the NPL(pairs) statistic identifies several chromosomal regions with suggestive evidence of linkage. Of particular interest is evidence for coincident mapping of each trait T2DM, MS, CVD1, CVD2, CCP, and CarCP to three different regions. All 6 traits map to 3q13 (peak 128 cM, nearest marker D3S4523) with LOD scores ranging between 1.15-2.51 with the highest LOD score with CVD2. A second locus is at 4q31 (peak at 146 cM, nearest marker D4S1625) with LOD scores ranging between 0.90-2.41 with the highest LOD score with T2DM. A third locus is on chromosome 14 (peak 23 cM, nearest marker ATA77F05) with LOD scores ranging between 0.58-2.31 with the highest LOD score with MS. A fourth locus at 86-94 cM on chromosome 3 shows evidence of linkage to T2DM/MS and CVD2/CCP with the highest LOD= 2.71 for CVD2. This comprehensively phenotyped sample of families provides the ability to map novel combinations of traits of great biomedical interest.

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Pleiotropic QTLs on chromosomes 5p13-p15, 16q22 and 19p13 influencing blood pressure and body composition measures: The NHLBI Family Heart Study. *M.F. Feitosa[1], M.A. Province[1], K.E. North[2], D.K. Arnett[3], R.H. Myers[4], J.S. Pankow[5], P.N. Hopkins[6], I.B. Borecki[1].* 1) Washington Univ, St. Louis, MO; 2) Univ North Carolina, Chapel Hill, NC; 3) Univ Alabama, Birmingham, AL; 4) Boston Univ, Boston, MA; 5) Univ Minnesota, Minneapolis, MN; 6) Univ Utah, Salt Lake City, UT.

Obesity is a risk factor for hypertension, and both are predictors of coronary heart disease. To identify locations of quantitative trait loci (QTLs) influencing variation of both blood pressure (systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP)) and body composition (fat free mass (FFM), percent of body fat (PBF), waist circumference, waist-to-hip ratio and body mass index (BMI)), bivariate variance component linkage analyses were employed on 2,766 subjects within 397 Caucasian pedigrees from the NHLBI Family Heart Study using 402 polymorphic markers. African American sample will be analyzed once genotyping has been conducted. All phenotypes were adjusted for age and sex effects. Bivariate LOD scores of 3.53 for FFM-SBP and 2.93 for FFM-MAP were found on chromosome 5p13-p15. These bivariate linkage peaks were substantially higher than the corresponding univariate linkage results for these traits at this location (FFM: LOD = 1.48, SBP: LOD = 0.88; MAP: LOD = 1.23). On chromosome 16q22, LODs of 3.08 for BMI-MAP and 2.85 for BMI-SBP were detected, which were higher than the respective univariate LODs (BMI: LOD = 1.00, SBP: LOD = 0.74; MAP: LOD = 0.77). Another pleiotropic QTL was observed on chromosome 19p13. The bivariate LODs of 3.01 for PBF-DBP and 2.80 for PBF-MAP were higher than the univariate LODs (PBF: LOD = 1.32, DBP: LOD = 2.55; MAP: LOD = 2.22). Despite evidence of linkage on 5p13-p15, 16q22 and 19p13 from several studies for metabolic syndrome phenotypes, pleiotropic QTLs influencing blood pressures and body composition measures have been largely unexplored. Chromosomes 5p13-p15, 16q22 and 19p13 harbor several plausible candidate genes, including NPR3, AGRP, HSD11B2, CHD10, CHD12, CHD6, CHD1, CHD8, CHD15, FOXC2, RETN, INSR and LDLR.

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Genome-wide scan for hypertension: Taiwan Young-Onset Hypertension Genetic Study. *W.H. Pan[1], C.M. Chung[1], C.S.J. Fann[1], J.W. Chen[2], C.L. Hsu[1], Y.S. Jong[3], H.M. Lo[3], F.M. Ho[3], C.S. Kang[4], C.C. Chen[5], H.C. Chang5[5].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 3) Tao-Yuan General Hospital Department of Health, Taiwan; 4) Min-Sheng Hospital, Taoyuan, Taiwan; 5) Lin-Shin Hospital, Chung-Li, Taiwan.

Efforts to map hypertension genes have been proven difficult without large sample size and phenotype with strong genetic component. Therefore, we carried out a multi-centered genome-wide linkage study with young-onset hypertension as the phenotype. We have recruited 1447 individuals, including 675 affected patients from 464 families. There were 253 affected sibpairs with hypertension and 172 pairs with young-onset hypertension, respectively. The maker set of deCODE genetics that defines a ~10 cM resolution was typed. Evidence of linkage was assessed with a non-parametric linkage method using GENEHUNTER. Two point linkage analyses were calculated, using the SIBPAL program. Indication of linkage signals were demonstrated consistently by two methods for regions on chromosome 1, 2, 3, 4, 6, 7, 8, 19, and 22. Among them, a region on chromosome 2 (140-170 cM) with a NPL score over 2 overlaps with findings of several other studies. There are several other novel positions identified by our study, including three with NPL score over or close to 1.5. In the second stage, we performed fine mapping with 32 markers spanning 40 cM (115-155) on chromosome 2. The NPL score was raised from 2.06 to 2.21. We then used tag SNPs selected from public HapMap database for all genes in this candidate region to pin down the hypertension susceptibility gene. Genotyping for a total of 20 SNPs in ten genes was obtained using mass spectrometer-based detection implemented on a Sequenom MassARRAY system. Transmission disequilibrium test analysis implemented in GENEHUNTER was performed for fine mapping. A significant association was found between hypertension and a haplotype constructed by 3 markers flanking the region for POLR1B, CHCHD5, SLC20A and FLJ40629 (P=0.000037). SLC20A, a sodium-dependent phosphate transporter, is the most likely culprit gene for hypertension.

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From genomewide scan to QTL fine mapping for ACE activity with young-onset hypertension patients. *R.Y. Wang[1], C.M. Chung[2], C.S.J. Fann[2], J.W. Chen[3], Y.C. Liu[2], Y.S. Jong[4], H.M. Lo[4], C.S. Kang[5], C.C. Chen[6], H.C. Chang[6], W.H. Pan[2].* 1) School of Public Health, China Medical University, Taichung, Taiwan; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 3) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 4) Tao-Yuan General Hospital Department of Health, Taiwan; 5) Min-Sheng Hospital, Taoyuan, Taiwan; 6) Lin-Shin Hospital, Chung-Li, Taiwan.

Genetic variation in ACE has been associated with hypertension and related diseases. The purpose of this study was to map QTL for ACE activity, a highly heritable intermediate phenotype for blood pressure or hypertension. We recruited 1168 individuals from 305 young-onset (onset age 40 years) hypertension pedigrees. The marker set of deCODE genetics that defines a ~10 cM resolution in human index map was typed for the study subjects. Normalized ACE activity as a quantitative trait was analyzed using SOLAR package. The heritability of ACE activity trait was estimated 0.52 after covariates were adjusted. Two-point analysis showed the highest LOD score at 89.6 cM (LOD=5.28 for D17S957) and multipoint linkage analysis at 83 cM (LOD=4.76) of chromosome 17, respectively. We also identified other loci with LOD3 located at 143.91 cM on chromosome 9 (LOD=5.22 for D9S1863) and at 68.82cM on chromosome 22 (LOD=3.73 for D22S1169) by two-point analysis and at 187 cM on chromosome 1 (LOD=3.29) and at 144 cM on chromosome 9 (LOD=4.59) by multipoint analysis. These results support that the ACE gene or nearby loci on 17q were among the strongest candidate genes influencing the ACE activity. Subsequently, 23 informative SNPs marker spanning the ACE locus were genotyped. FBAT and TRANSMIT softwares were performed to relate high ACE activity (80th percentile) to individual SNPs and haplotype blocks constructed with 3-consecutive SNPs. Individual SNPs and haplotype blocks nearby the 3 end of ACE locus in intron 20-24 have the largest (-log(P)=14.43). We have disclosed multiple QTLs for ACE activity. Our data suggest that the QTL on ACE gene locate between intron 20 to 24. This finding may lead to understanding on how ACE expression is regulated.

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Genomic localization of a locus on chromosome 4 linked with variation of HDL Cholesterol. *Z.Y.H. Wong, K.J. Scurrah, A. Lamantia, S.B. Harrap.* Department of Physiology, University of Melbourne, Victoria 3010, Australia.

Plasma concentrations of high-density lipoprotein cholesterol (HDL-C) are inversely correlated with the risk of cardiovascular disease.[1] Genetic factors account for 57% of the variance of HDL-C.[2] In this study we undertook a 3 stage genotyping analysis in white healthy adult families from the Victorian Family Heart Study.[2] In 274 adult sibling pairs of average age 24 year a genome-wide scan revealed a locus on chromosome 4 at 170 cM with suggestive genome-wide evidence (Z score = 3.34, 1 Z confidence interval: 34 cM) of linkage to HDL-C. Linkage results were not materially affected by adjustment for covariation with age, sex, weight, height, and body mass index. In 233 of the sibling pairs and their parents we undertook fine mapping of the chromosome 4 locus with 11 microsatellite markers with average intermarker distances of 1.34 cM. This analysis based on genotypes and phenotypes from both generations strengthened our preliminary findings with a peak probability (Z score = 3.9) at 169 cM (1 Z estimated confidence interval: 17 cM). Our linkage data strongly suggests a genetic location around 169 cM on chromosome 4q32.3 is linked with HDL-C. In this region exists the gene encoding the carboxypeptidase E. The third stage involves association tests of single nucleotide polymorphisms (SNPs) and haplotypes in and around candidate gene(s) or selected loci of this region with HDL-C to elucidate the functional variants. Groups of genetically unrelated males & females having high (mean 2.37 mmol/L) or low (mean 0.77 mmol/L) HDL-C levels were mapped initially with 18 SNP markers spanning 33.4 Kb. 5 SNPs showed evidence of association with HDL-C with and/or without genotype-sex interactions. These preliminary data demonstrated the feasibility of our scientific strategy and methodology, and add further credence to this specific locus on chromosome 4q32.3 for HDL-C control.

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Developing a Laboratory Information Management System (LIMS) in a high-throughput genotyping facility. *C. Boyce, J. Romm, B. Craig, C. Bark, M. Zilka, Y. Sun, M. Barnhart, C. Boehm, R. King, J. Goldstein, E. Pugh, K. Doheny.* Center for Inherited Disease Research, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) is a centralized facility established to provide genotyping and statistical genetics services for investigators seeking to identify genes that contribute to human disease. As the demand for services increased, CIDR was required to alter the way operations were performed. Included in this need for change was the migration from the Applied Biosystems (AB) 3700 to the AB 3730 and GeneMapper software. Due to the increase in capacity and change of platform, CIDR needed to implement a new LIMS to replace the existing LIMS that had been developed in-house over the years. A new data transfer system, customization of GeneMapper software, and a web interface between CIDRs Project Management and the Principal Investigator (PI) was also required. CIDR spent about two years working with the AB Professional Services group (formerly known as Rapid Integrated Solutions) to design, develop, test and integrate a new LIMS system, custom WebLIMS, and data release process, all driven by a new relational database, SQL*LIMS, LSL and other custom functionality. Based on CIDRs experience developing a LIMS in-house versus hiring an outside contractor to develop a LIMS, there are several conclusions that can be made. From a constructive viewpoint, there was a steep learning curve for CIDR staff to learn the core capabilities of the new system and for CIDRs Informatics group to learn to manage, maintain and further customize the new system. The database administrative duties are much more complex and the programmers must modify source code written by others. However, the new LIMS is more highly integrated and relational than the existing in-house LIMS, tracking and record keeping is unsurpassed, the system is more organized and it allows for better lab and workflow management. The overall experience was satisfactory and the new well-integrated LIMS has been implemented successfully and is currently being used for production genotyping.

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Using a Utah pseudo isolate population to identify predisposition genes for prostate cancer. *L.A. Cannon-Albright, J. Farnham, N.J. Camp.* Dept. Medical Informatics, Univ Utah Sch Medicine, Salt Lake City, UT.

Using a retrospective approach to study design, we have identified multiple pseudo isolate populations in Utah, originating from the Utah Mormon pioneers (Cannon-Albright et al., in press). We have defined several birth-country pseudo isolates in the Utah Population Dababase (UPDB), selecting Utah Mormon pioneer founders with a specific birthplace from among all pioneers, and restricting the pseudo isolate population to include only descendants of the founder-matings, and their descendants. That is, from a selected set of founders, and allowing no immigration, we identify a smaller, pseudo isolate sub-population . We hypothesize that pedigrees identified in these pseudo isolate populations represent a genetically homogeneous, informative resource for complex disease predisposition gene mapping. We present an example of a single pseudo England isolate high risk prostate cancer pedigree, which alone provides compelling evidence for linkage of prostate cancer predisposition on chromosome 18. We have identified 8 high risk England pseudo isolate prostate cancer pedigrees with 6 or more prostate cases representing a significant excess of prostate cancer among the isolate descendants of the pedigree founder. One of these 8 pedigrees was previously genotyped in a genome wide linkage study for prostate cancer. The England isolate pedigree contained 6 purely English prostate cancer cases, with 5 of the cases genotyped or inferred from spouse and offspring. Four regions on chromosomes 1, 5, 13 and 18 showed evidence for linkage in this English pedigree (multipoint hetLOD > 1.0), all with a dominant model, with the chromosome 18 evidence being the most compelling (multipoint LOD>1.70, $p<0.002$ for several sequential markers). All 5 of the genotyped prostate cancer cases in the England isolate pedigree share a chromosome 18 haplotype. Subsequent genotyping of prostate cases in 2 additional Utah English isolate pedigrees has identified haplotype-sharing in the same region.

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Estimating joint informativeness of markers in a haplogroup. *H. Lee, X. Sheng, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

With more genetic markers available, closely linked markers are jointly utilized as a haplogroup. When markers in such a haplogroup are to be used together, inter-locus allelic dependency needs to be considered in computing haplotype frequencies. Since this dependency is influenced by mutation rate, population demographic history, and physical distance between the markers, any attempt of estimating haplotype frequency, and haplotype diversity (as an index of joint informativeness) using analytical method becomes computationally involved. Alternatively, when haplotypes are identifiable by direct laboratory methods, such population genetic parameters are estimated empirically using the gene count method. However, as the number of markers in a haplogroup increases, the number of possible haplotypes quickly outnumbers the sample size, making the estimated parameters more dependent on the sample size. Consequently, with the fixed sample size, increased number of markers does not necessarily lead to an elevated joint informativeness. In order to investigate the optimal number of markers that would produce the maximum informativeness given the conventional DNA database size, we analyzed haplotype data consisting of 19 Y-chromosomal microsatellites, from US black (N=260) and US Caucasian (N=244) populations. The results show that, in the US black population, only six markers were needed to reach the haplotype diversity of 0.999, while additional 10 more markers were necessary to elevate the value into 0.9997. Sequential addition of markers, ordered by their gene diversity did not necessarily produce the optimum improvement in joint informativeness, but in general, this approach provided better results than randomly selected set of markers. Data from the US Caucasians demonstrated the similar results. Our study indicates that, extending over conventional minimal set of nine Y-chromosomal markers will only marginally improve the joint informativeness. Also, for a set of closely linked markers, determining reference database size requires different guidelines from that of autosomal markers. (Research supported by a federal training grant on research in biothreat agents).

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Genome wide linkage scan of 758 affected sibling pair families with rheumatoid arthritis using the Illumina Linkage IV set of ~5,600 SNP markers. *C.I. Amos[1], W. Chen[1], A.T. Lee[2], M.F. Seldin[3], L.A. Criswell[4], D.L. Kastner[5], E.F. Remmers[5], W. Li[2], M. Kern[2], P.K. Gregersen[2].* 1) Dept Epidemiology,MD Anderson Cancer Ctr, Houston, TX; 2) NSLIJ Research Institute, Manhasset, NY; 3) Rowe Program in Human Genetics, UC Davis, CA; 4) Department of Medicine, UCSF, San Francisco, CA; 5) NIAMS/NIH, Bethesda, MD.

The North American Rheumatoid Arthritis Consortium (NARAC) has assembled a large collection of affected sibling pairs with rheumatoid arthritis (www.naracdata.org). In addition to a strong signal in the HLA region, previous microsatellite genome screens on 512 of these families have revealed modest evidence linkage (LOD 1.5-2.5) at several other chromosomal locations. We have now performed a complete genome wide linkage scan using ~5,600 informative SNP markers on 758 NARAC families containing 931 affected sibling pairs. Linkage analysis was carried using a call to MERLIN from SNPLINKer. Allowance for linkage disequilibrium between SNP markers (D<0.7) required the removal of approximately 17% of the genotypes, and eliminated an apparently spurious linkage peak at the extreme telomeric portion of chromosome 6q. The data confirm a very strong and broad linkage peak on chromosome 6p, centered on the HLA region (max NPL score =18.9 at 32.9 cM), but extending from 13 cM on 6p out to 80cM on 6q with LOD scores >3. This 67 cM linkage interval suggests the likely involvement of multiple loci on this chromosome in RA susceptibility. In addition, previously identified regions with very modest evidence linkage on chromosomes 4, 5 and 11 now show more compelling evidence of linkage (LOD scores > 2.8, >2.8 and >3.5 respectively). Finally, we have been able to narrow a previously observed linkage peak on chromosome 18q to a region around 52cM, consistent with the results of a recent dense association mapping study of this region (Remmers et al, abstract submitted). These results greatly refine regions of linkage we previously have reported and demonstrate that significant additional linkage information can be extracted from multiplex families by using a suitably dense set of informative SNP markers across the genome.

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Fine-mapping of a diabetic nephropathy locus on 3q24-q27 and association analysis with the candidate gene *PRKCI*. *J.K. Wolford[1], M.P. Millis[1], K.A. Yeatts[1], S. Rais[1], R.G. Nelson[2], W.C. Knowler[2], R.L. Hanson[2].* 1) TGen, Phoenix, AZ; 2) NIDDK, Phoenix, AZ.

Diabetic nephropathy is the major cause of end-stage renal disease (ESRD). In the Pima Indians of Arizona, who have very high rates of ESRD attributable to diabetes, a genome scan found evidence for linkage for nephropathy on 3q24-q27 (LOD=2.14). To fine-map the linkage interval, we genotyped 14 microsatellite markers in addition to the 34 from the original genomic scan, yielding a final mean map density (SE) of 2.50.7 cM. The information content was 0.57-0.67 with the original markers and increased to 0.67-0.73 with the additional markers. Linkage analysis was performed using the S_{all} function in GENEHUNTER-PLUS in 98 diabetic sibpairs concordant for nephropathy in whom linkage had been originally detected. In the original analyses, the strongest evidence for linkage (LOD=2.14) was found between markers *D3S1763* and *D3S3053* at map location 181 cM. With the additional markers, the evidence for linkage on 3q increased to LOD=2.63. Association analyses of candidate genes in this region are being conducted in 107 cases with diabetic ESRD and 108 controls with diabetes >10 years and no evidence of nephropathy and in the families from the original linkage study (148 diabetic individuals with nephropathy and 458 without nephropathy in 264 sibships). Initial analyses focused on the protein kinase C-iota (*PRKCI*) gene. We sequenced all 18 exons, exon-intron boundaries and 2 kb of the 5 regulatory region in 36 Pimas and identified 13 SNPs. None of the SNPs showed a statistically significant association in either study group (P>0.05). The strongest results were seen with SNP133631, where the minor allele had a frequency of 0.22 in cases and 0.12 in controls (OR=1.61 per copy of the minor allele, 95% CI=0.98-2.63, p=0.06); the corresponding OR in the family study was 1.41 (95% CI=0.88-2.27, p=0.15): these ORs correspond to an effect of ~1% on the variance in liability to nephropathy, which is unlikely to explain the linkage signal. These analyses support the presence of a locus for diabetic nephropathy at 3q24-27, but suggest that variation in *PRKCI* does not explain this finding.

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Fine-mapping and characterization of the mouse autoimmune diabetes locus Idd21.1. *T. Merriman, S. Hook, J. Hollis-Moffatt.* University of Otago, Dunedin, New Zealand.

Mouse *Idd21.1*, on distal Chr18, maps to a region orthologous to human *IDDM6*. The aims of this work were to fine-map *Idd21.1* and to test whether *Idd21.1* operates through a T lymphocyte-mediated immune pathway.

Beginning with the congenic strain NOD.ABH-(*D18Mit8-D18Mit4*) (with 11cM of distal Chr18 ABH diabetes-resistant DNA on the non-obese diabetic (NOD) background), two novel mouse strains were created (NOD.ABH-(*D18Mit8-D18Mit46*) and NOD.ABH-(*D18Mit8-D18Mit216*), containing 3 and 9cM of distal Chr18 ABH DNA, respectively) and diabetes incidences determined. NOD.ABH-(*D18Mit8-D18Mit46*) had a reduced diabetes incidence vs NOD (57% and 85% at 7 months respectively, $P<0.0001$), providing evidence for *Idd21.11* in the 47-50cM region. Given there was no significant difference between NOD.ABH-(*D18Mit8-D18Mit46*) and NOD.ABH-(*D18Mit8-D18Mit216*) (57% and 63% respectively, $P=0.24$), there was no evidence for an Idd locus in the 50-56cM interval. NOD.ABH-(*D18Mit8-D18Mit216*) had an increased diabetes incidence vs NOD.ABH-(*D18Mit8-D18Mit4*) (63% and 48% respectively, $P=0.02$), providing evidence for *Idd21.12* in the 56-58cM interval. A third congenic strain, NOD.ABH-(*D18Mit8-Madh2*), provided further support for *Idd21.11* (67% diabetes incidence, $P=0.0001$ vs NOD). A fourth strain, NOD.ABH-(*D18Nds1-D18Mit4*), provided further support for *Idd21.12* (75% diabetes incidence, $P=0.01$ vs NOD). Thus, *Idd21.1* consists of two subloci (*Idd21.11* and *Idd21.12*).

Adoptive transfer of splenocytes from NOD and NOD.ABH-(*D18Mit8-D18Mit4*) into lymphocyte-deficient NOD.*scid* and NOD.*scid*.ABH-(*D18Mit8-D18Mit4*) mice was done. The NOD splenocytes were equally efficient at transferring diabetes to NOD.*scid* and NOD.*scid*.ABH-(*D18Mit8-D18Mit4*) ($P=0.24$), suggesting that *Idd21.1* does not influence intrinsic resistance of -cells to immune attack. NOD.ABH-(*D18Mit8-D18Mit4*) splenocytes were more efficient at transferring diabetes to NOD.*scid* than to NOD.*scid*.ABH-(*D18Mit8-D18Mit4*) ($P=0.002$), consistent with the hypothesis that *Idd21.1* may regulate diabetes through interaction between the adaptive and innate immune systems.

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Bivariate linkage analysis of TNF-alpha and Body Mass Index in Type 2 Diabetes. *J. Zhou, G. Chen, A. Adeyemo, Y. Chen, C. Rotimi.* Pharm, Natl Human Genome Ctr, Howard Univ, Washington, DC.

Bivariate linkage analysis of TNF-alpha and Body Mass Index in Type 2 Diabetes Guanjie Chen, Adebowale A. Adeyemo, Jie Zhou, Yuanxiu Chen, Charles Rotimi National Human Genome Center at Howard University College of medicine, Washington D.C Obesity is one of the biggest generators of silence inflammation. The cytokine TNF-alpha produced by adipocytes is a key component of the insulin - resistant state in obesity and contribute to the development of type 2 diabetes. Bivariate linkage analyses of correlated traits provide greater statistical power to identify genetic loci with effects too small to be detected in single trait analyses. We conducted genome wide bivariate analyses of TNF-alpha and Body Mass Index (BMI) using a sample of sib pairs (321 sibling pairs and 36 half sibling pairs) affected with T2DM from the Africa America Diabetes Mellitus (AADM) study. Participants were genotyped with a panel of 372 autosomal microsatellite markers with an average intercal of 9cM and multipoint linkage analysis done using the variance components approach. Three suggested linkage evidence regions (two regions, LOD = 3.02 (142cM), near marker D2S1334 and LOD = 3.33 (173cM), at marker D2S1776 on Chromosome 2, and one region with LOD = 3.23 (131cM), near marker D11S912 on Chromosome 11) were identified and one tentative linkage evidence region (LOD = 2.19 (146cM), near marker D1S1631 on chromosome 1) was identified. By conducting bivariate linkage analyses for each measure of TNF-alpha level with a measure of obesity (BMI), the combination provides additional advantage in searching for TNF-alpha linkage regions in type 2 - diabetic patients.

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A dense SNP map of human chromosome 20q13.1: linkage disequilibrium, haplotype analysis, and association with type 2 diabetes in a 6.0 Mb interval. *D.W. Bowden, J..L. Bento, M. Zhong, C.D. Langefeld, B.I. Freedman, J.C. Mychaleckyj.* Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC.

Human chromosome 20q12-13.1 is linked to type 2 diabetes mellitus (T2DM) in multiple studies. To identify T2DM susceptibility gene(s) in this region, a SNP map has been constructed across a 5.83 Mb region (HG Build 35: 41.4 - 47.2 Mb), which contains 75 genes including the candidate diabetogenic genes, adenosine deaminase (ADA), hepatocyte nuclear factor 4 alpha (HNF4A) and glucose transporter 10 (GLUT10). Verified SNPs from the NCBI dbSNP and Appelera/Celera Discovery System databases, with minor allele frequency ranging from 0.04-0.49, were genotyped using a mass spectrometry based genotyping system and placed on the map at an average density of 1 SNP per 17 kb. A total of 337 SNPs were genotyped on 310 Caucasian controls and 300 Caucasian cases with T2DM and end stage renal disease (ESRD). Inter-SNP linkage disequilibrium (LD) was calculated using D as a measure of LD. 226 SNPs (67% of all SNPs on map) fall into 83 LD blocks. Evaluation of LD across the map comparing T2DM cases to controls reveals higher levels of LD in the case population. Within regions of significant high LD (D 0.70, P-value 0.05), haplotypes were constructed using HAPLO.SCORE and Dandelion. In single SNP analysis 53 SNPs (15.7% of 337 total) showed nominal evidence of association in one or more point marker tests of association (P 0.05), and 9 SNPs (0.03%) retained test significance at P 0.005. These latter SNPs lie in 4 perigenic regions, including SLC12A5 (potassium-chloride cotransporter), NCOA5 (nuclear receptor coactivator 5), CDH22 (cadherin-like 22), and PRex1 (phosphatidylinositol 3,4,5-triphosphate-dependent RAC exchanger 1). After adjustment for multiple tests at a Family Wise Error Rate (FWER) < 0.05, two T2DM-associated SNPs in the PRex1 region retained map-level significance. In addition, LD haplotype blocks encompassing the associated single SNPs in SLC12A5/NCOA5 and PRex1 regions were significantly associated with the T2DM (P<0.005 for both).

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Fine mapping a locus for early-onset type 2 diabetes in African American families. *M.M. Sale[1,2], T.S. Leak[1], C.D. Langefeld[3], C.J. Valis[3], C.J. Gallagher[1,4], B.I. Freedman[2], D.W. Bowden[1,4].* 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 4) Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC.

To identify susceptibility loci for type 2 diabetes mellitus (T2DM) and diabetic nephropathy in African Americans, a genome wide screen was performed on 638 African American affected sib-pairs (ASPs) with T2DM from 247 families; 206 ASP from 166 families were concordant for end-stage renal disease (ESRD)/nephropathy. Ordered subsets analysis (OSA) revealed a linkage peak on chromosome 7p in the subset of T2DM families with an early age of diagnosis (29% of pedigrees, max. LOD=3.85, p=0.0034 for the change in LOD score). T2DM-ESRD subsets with lower BMI (64% of pedigrees, max. LOD=3.93, p=0.01) and longer duration to ESRD (37% of pedigrees, max. LOD= 3.59, p=0.01) also showed evidence for linkage. Nonparametric linkage regression (NPL) analyses provided evidence for interactions with early age at T2DM diagnosis (p=0.0039), duration of diabetes to onset of ESRD (p=0.0281), and low BMI in T2DM-ESRD ASP (p= 0.0143). We genotyped eleven additional polymorphic markers in the region of interest, with an average spacing of 4 cM, in the same pedigrees. OSA supported evidence of linkage at 28 cM on chromosome 7p, closest to D7S3051, in early-onset T2DM families (21% of pedigrees; max. LOD= 3.61, p=0.0025; mean age at diagnosis 29.5 3.0 years). In T2DM-ESRD families, the evidence for linkage in subsets with lower BMI (64% of pedigrees; max. LOD= 2.20, p=0.11) and longer duration of diabetes before onset of ESRD (56% of pedigrees; max. LOD=2.81, p=0.20) diminished. There are several plausible diabetes candidates in this region, including interleukin-6, neuropeptide Y, and phosphodiesterase 1C. Refining the linkage peak on chromosome 7p provides substantial evidence of susceptibility gene(s) for early-onset T2DM in our African American sample.

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Evidence for multiple distinct obesity/type 2 diabetes mellitus loci under a single peak of linkage on chromosome 11q23: replication in ethnically diverse populations. *L. Baier[1], Y. Muller[1], L. Ma[1], M. Traurig[1], P. Kovacs[2], A. Bottner[2], S. Stone[3], K. Timms[3], C.J. Groves[4], M. McCarthy[4], M. Erdos[5], K. Conneely[6], M. Boehnke[6], K. Miyake[7], L. Atwood[8], L. Johansson[9], M. Ridderstale[9], W. Knowler[1], R. Hanson[1], C. Bogardus[1].* 1) NIDDK/NIH, Phoenix, AZ; 2) Leipzig, Germany; 3) Salt Lake City, UT; 4) Oxford, UK; 5) Bethesda, MD; 6) Ann Arbor, MI; 7) Chicago, IL; 8) Boston, MA; 9) Malmo, Sweden.

A prior genomic linkage scan in Pima Indians indicated an obesity susceptibility locus on chromosome 11q23-24 (LOD=3.6). There was also evidence that the same genomic region contained a susceptibility locus for type 2 diabetes mellitus (T2DM)(LOD=1.7). Bivariate linkage analysis for the combined phenotype diabesity gave the strongest evidence for a disease locus (LOD= 5.2). The region of linkage spans 24 Mb. To narrow this region, single nucleotide polymorphisms (SNPs) are being genotyped at a 10 kb density, across the entire 24 Mb, for linkage disequilibrium (LD) mapping. To date, more than 2000 SNPs have been genotyped and tested for association with either BMI or T2DM in 1229 Pima Indians. LD mapping using this genotypic data shows three distinct regions (Regions 1-3), each spanning approximately 500 kb, that contain multiple SNPs significantly associated ($p<0.01$) with BMI in Pima Indians. SNPs within two of these regions have been further genotyped in other, ethnically diverse populations. SNPs in Region 1 are modestly associated with BMI in two groups of Finns (FUSION study and Malmo), USA Caucasians (Framingham) and German Caucasian children (Leipzig School Children) (all $p<0.05$ after adjusting for age and sex). SNPs in Region 1 are also associated with T2DM in Pima Indians ($p<0.001$), Mexican Americans (Starr County; $p<0.05$) and Finns (Helsinki; $p<0.02$). SNPs in Region 2 are associated with BMI in two Caucasian populations (Framingham and Utah; $p<0.001$ and $p<0.05$, respectively, after adjusting for age and sex) and are also associated with T2DM in Utah Caucasians. In contrast, none of the SNPs that were tested from either region were associated with BMI or T2DM in Caucasians from the UK. We are currently attempting to replicate associations in Region 3.

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Fine-mapping the MKS3 locus for Meckel-Gruber syndrome. *U.M. Smith[1], N.V. Morgan[1], E.R. Maher[1], P. Cox[2], C. Bennett[3], T. Attie-Bitach[4], C.A. Johnson[1].* 1) Medical and Molecular Genetics, University of Birmingham, Birmingham, UK; 2) Dept. of Pathology, Birmingham Womens Hospital, Birmingham, UK; 3) Clinical Genetics, St. James Hospital, Leeds, UK; 4) Departement de Genetique et Unite INSERM U-393, Hopital Necker-Enfants Malades, Paris, France.

Meckel-Gruber syndrome (MKS) is a lethal autosomal recessive developmental disorder characterised by encephalocele, large multicystic kidneys, polydactyly and fibrocystic changes to the liver. The incidence of MKS varies between different populations, and MKS shows genetic heterogeneity with three known loci. *MKS1* is on chromosome 17q21-q24, *MKS2* on chromosome 11q13 and *MKS3* on chromosome 8q. Here we report the fine-mapping of *MKS3*. We have used a new cohort of Pakistani consanguineous families to redefine the critical interval for *MKS3*. Linkage to *MKS1* and *MKS2* was formally excluded with microsatellite markers. Our cohort of patients showed distinct phenotypic similarities, such as cleft palate and low set ears, in addition to the standard diagnostic criteria. Six families underwent a combination of Affymetrix 10k SNP chip genome-wide linkage analysis and microsatellite linkage analysis. This yielded a two-point cumulative LOD score of 5.24 for = 0 at marker D8S1988, corresponding to the centre of the region. A family that is multiply consanguineous with two affected second cousins, has narrowed the critical interval due to an obligate recombination in an unaffected sibling. Further fine-mapping using novel microsatellites has reduced the interval to chromosome 8q21.2-23, between secure boundaries. We are in the process of sequencing candidate genes in this interval for causative mutations.

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Linkage analysis in families with generalized epilepsy with febrile seizures plus. *A.M. Madsen, Y. Wang, M.R. Winawer, W.A. Hauser, C. Barker-Cummings, R. Ottman.* Sergievsky Ctr, Columbia Univ, New York, NY.

Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome with variable phenotypes, including typical febrile seizures (FS), febrile seizures plus (FS+) (i.e., FS persisting beyond age six or accompanied by afebrile generalized-tonic seizures), and generalized and focal epilepsy. Mutations in SCN1B (19q13), GABRG2 (5q31), SCN1A (2q24) and SCN2A (2q24), have been identified in GEFS+ families. We sought to evaluate evidence for mutations in these known genes, and to localize other genes for the syndrome. We selected, from a set of 88 families containing 2 individuals with idiopathic or cryptogenic epilepsy collected for genetic linkage analysis, a subset of 16 families that appeared consistent with GEFS+ (i.e., those containing two or more individuals with febrile seizures). We typed all available family members at 390 microsatellite markers spaced at an average of 10cM intervals across the genome. The families contained an average of 13.5 (range 3-32) genotyped individuals, of whom 5.9 (range 3-14) were affected with idiopathic or cryptogenic epilepsy or febrile seizures. In initial analyses, we assessed the evidence for linkage to markers within 5cM of the previously identified genes, assuming a dominant model with 75% penetrance. In analyses of all families combined, linkage was excluded at theta < 0.10 for each of the markers. In analyses of individual families, however, two families gave suggestive linkage evidence (lod scores of 1.67 and 1.01 respectively) for D2S1776, located 4cM telomeric to SCN1A. Each of them contained 13 or more affected individuals, five or more of whom had FS+. None of the other families had a lod score exceeding 1.0 for any of the markers near the other three genes. These results suggest that SCN1A mutations may underlie the GEFS+ phenotype in two of the 16 families; mutation screening in these families is warranted. In the remaining families, mutations in the four previously identified genes are less likely, and we are continuing to screen for linkage in other chromosomal regions.

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Screening for DFNB1, DFNB2, DFNB3, DFNB4, DFNB9 and DFNB21 loci in the Iranian patients with autosomal recessive non-syndromic hearing loss. *Y. Riazalhosseini[1], N.C. Meyer[2], A. Daneshi[3], M. Mohseni[1], N. Bazazzadegan[1], M. Avenarius[2], P. Imanirad[1,4], M. Farhadi[3], K. Kahrizi[1], R.J.H Smith[2], H. Najmabadi[1].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences,Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 3) Department of Otolaryngology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran; 4) Molecular-Cellular Biology Department, Khatam University, Tehran, Iran.

Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common form of severe inherited hearing impairment. To date, at least 45 loci for ARNSHL have been identified, indicating it as an extremely heterogeneous disorder. These loci are referred to as DFNB loci. Although, mutations at the DFNB1 locus (including GJB2 and GJB6 genes) are the most common cause of ARNSHL in populations originating from Northern Europe, we have shown that mutations in GJB2 and the (GJB6-D13S1830) do not play a significant role in the etiology of deafness in Iran. In this study, we assess the contributions made by other loci to the ARNSHL genetic load in Iran. We have selected 50 consanguineous families with normal GJB2 and GJB6 alleles to be screened for linkage to the DFNB2, DFNB3, DFNB4, DFNB9 and DFNB21 loci. Linkage analysis is applied using, in average, 3 short tandem repeat (STR) markers for each locus. These loci have been excluded in 18 families but two families; each has been localized to DFNB4 and DFNB21. We are screening these families for mutations in SLC26A4 and TECTA, respectively. Our results suggest that other loci may have the major causative roles in ARNSHL in Iran. This hypothesis will be confirmed by including more families and screening additional loci.

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Mapping a third locus for familial TAAD (TAAD3) using samples from a single family with multiple affected individuals and determining the contribution of this locus to familial disease. *N. Avidan[1], V. Tran Fadulu[1], J. Chen[1], J. Yuan[1], A. Braverman[3], R. Yu[2], S. Shete[2], D.M. Milewicz[1].* 1) Department of Internal Medicine, The University of Texas Medical School at Houston, Houston, TX; 2) Department of Epidemilogy, The University of Texas M.D. Anderson Cancer Center, Houston, TX; 3) Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO.

Familial thoracic aortic aneurysms and dissections (TAAD) is an autosomal dominant condition with reduced penetrance and variable expression. Genes predisposing to TAAD have been mapped to 5q13-14 (TAAD1), 11q23 (FAA1), and 3p24-25 (TAAD2). We collected a large family, TAAD216, with a unique phenotype of dissections involving both the ascending and descending aorta and young age of onset in women. The disease phenotype was not linked to any of the known loci for familial TAAD or MFS, suggesting that the disease was due to a novel locus. The family consists of 134 individuals, 71 males and 63 females, with enough power to map a locus using only this family. We applied whole genome linkage analysis to 10K mapping SNP chip (Affymetrix) data, using a sliding window of 180-200 SNPs and the Allegro program on a sub-family with a pedigree of 24 individuals. Of these, 12 individuals were classified as affected, 5 as unaffected, and 7 as unknown. Parametric as well as non-parametric options were used to calculate the LOD scores. Initial analyses gave suggestive peaks on chromosomes 2 and 8 ranging from 1.5 to 2.0, and a peak on chromosome 15q that was over 4.0 with both parametric and non-parametric analysis. The three genomic regions were screened with microsatellite markers using 32 members from the extended family. No shared haplotype was found on chromosome 2 and 8, however a shared haplotype was found in 4 affected and 4 possibly affected individuals between D15S1046 and D15S130 with a maximum LOD score of 3 at marker D15S127. We screened 54 additional families with three or more affected individuals, and found 8 families linked to this locus, suggesting that this locus might contribute to 10-20% of familial TAAD. We propose to name the new locus TAAD3.

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Bayesian genome scan for cleft lip with or without cleft palate (CL/P). *M. Govil[1], J.C. Murray[1], M.L. Marazita[2], A. Lidral[1], L.L. Field[3], M. Arcos-Burgos[4], L. Moreno[1], C. Valencia[4], J. Risk[5], J.T. Hecht[6], K. Doheny[7], E. Pugh[7], C. Boehm[7], V.J. Vieland[1].* 1) U of Iowa; 2) U of Pittsburgh; 3) U of British Columbia, Canada; 4) U. of Antioquia, Colombia; 5) U of Liverpool, UK; 6) U of Texas, Houston; 7) CIDR.

Non-syndromic CL/P is one of the most commonly occurring congenital anomalies and has a complex etiology with birth prevalence that varies with geographic origin. There has been some recent progress in testing and identifying candidate genes and regions for CL/P, but given its complex etiology and epidemiology, large sample sizes across multiple populations and a variety of analytical approaches to address expected heterogeneity and identify multigenic causes are necessary. One such approach, the posterior probability of linkage (PPL), is designed to handle the complexity observed for CL/P. It integrates out the trait model parameters; allows explicitly for both intra- and inter-sample heterogeneity; and accumulates evidence for or against linkage across multiple heterogeneous datasets using Bayesian sequential updating. A two-point Bayesian 10 cM genome scan (markers genotyped by the Center for Inherited Disease Research) computing the PPL was carried out for 487 informative families drawn from 8 populations from 7 countries sequentially updated over population of origin. These families have been analyzed with multipoint parametric and nonparametric linkage approaches with several regions reaching genome-wide signifiance (see Marazita et al, this meeting). The following regions have two-point PPLs 10%: 1q23.3, 2p21, 6q16.1, 9q32, 12p12.3 and 12q13.13. While these 2-point PPL results support the multipoint HLOD and/or NPL results (Marazita et al), they also allow narrowing the overall region for further analysis to a total of 50 cM (approx.) with an 80% probability of a gene on at least one of chromosomes 1, 2, 6, 9 and 12. It is expected that multipoint PPL analyses currently underway will further reduce the region for fine mapping. NIH grants DE08559, DE09886, DE12472, DE13076, DE14667, DE016148, DE016215, RR-00084; CIDR contract N01-HG65403; Carver Charitable Trust.

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Dyslexia gene identifyed in an Icelandic sample. *H. Stefansson[1], A. Ingason[1], J. Halldorsson[2], G. Thorleifsson[1], T. Thorlacius[1], O. Rolfsson[1], G. Bjornsdottir[1], G. Sveinbjornsdottir[2], B. Richardsson[1], K. Kristjansson[1], A. Kong[1], U. Thorsteinsdottir[1], J.R. Gulcher[1], K. Stefansson[1].* 1) Dept Population Genomics, Decode Genetics, Reykjavik, Iceland; 2) National Blood Collection facility, Nóatún, Iceland.

Dyslexia is the most common learning disorder in individuals of normal intelligence affecting approximately 5% of those who have access to satisfactory educational resources. Contribution of genetic factors to the risk has been demonstrated by twin studies, segregation analysis and extended families have been used to identify loci linked to dyslexia. Current studies indicate locus heterogeneity as several different loci have been identified although only some have been replicated in independent cohorts. We have analyzed 82 Icelandic pedigrees where dyslexia segregates and our results are in line with previous studies indicating locus heterogeneity. No genome-wide linkage signal was achieved but supportive linkage was found to previously reported loci. Fine-mapping one locus, however, gave significant association to dyslexia in a cohort of 412 dyslectic individuals with a relative risk of 1.9 to a single marker ($p = 4.6x10-7$, PAR = 10.6% after correcting for relatedness) supported by surrounding markers and haplotypes. For 218 of the 412 genotyped dyslectic individuals both parents were genotyped. Of 62 informative transmissions studied 42 were transmissions of the at-risk variant to a dyslectic offspring ($p = 0.00014$).

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Genome-wide linkage scan of obsessive-compulsive disorder: significant evidence for susceptibility loci on chromosomes 3q21-27. *Y. Yao[1], JF. Samuels[1], V. Willour[1], A. Pinto[2], J. Knowles[3], J. McCracken[4], S. Rauch[5], D. Murphy[6], M. Riddle[1], B. Cullen[1], M. Grados[1], B. Greenberg[2], A. Fyer[2], P. Davis[5], J. Bienvenu[1], G. Nestadt[1].* 1) Dept Epidemiology, Johns Hopkins Univ, Baltimore, MD; 2) Brown University, Providence RI; 3) Columbia University, New York, NY; 4) Unversity of California at Los Angeles, CA; 5) The Massachusetts General Hospital, Boston, MA; 6) National Institute of Mental Health, Bethesda, MD.

Introduction: Obsessive-compulsive disorder (OCD) is a disabling psychiatric disorder characterized by obsessions and/or compulsions. Previous twin and familial aggregation studies support a genetic etiology. Here we present the first large scale model-free linkage analysis of OCD. Methods: 219 extended and nuclear families were assessed in the OCD Collaborative Genetics Study using standard diagnostic methods. A genome-wide microsatellite marker panel was genotyped at CIDR. Non-parametric linkage analyses were conducted using both broad (definite and probable diagnoses) and narrow definitions (definite only) of OCD. Results: Using a p-value of 0.01 as a cut point, significant linkage signals were observed on chromosome 3q (multipoint Kong and Cox LOD-ALL =2.62, p-value = 0.0003), 1p (multipoint Kong and Cox LODALL= 1.46, p-value =0.004), 7p (Kong and Cox LODpairs =1.97, p-value =0.0013), and 15p (Kong and Cox LOD-pairs =1.36, p-value =0.006). Empirical p values were calculated for the NPLall scores via simulation of 10,000 replicates using Merlin. These results strongly suggest evidence for linkage on chromosomes 3. Conclusions: We are currently pursuing candidate gene studies as well as SNP-based fine mapping in all significant regions, with particular focus on 3q21-27.

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Gemone wide linkage scan of aggressive prostate cancer: Results from the International Consortium for Prostate Cancer Genetics. *S.K. McDonnell[1], L. Dimitrov[2], D.J. Schaid[1], and the International Consortium for Prostate Cancer Genetics.* 1) Mayo Clinic College of Medicine, Rochester, MN 55905; 2) Wake Forest University School of Medicine Center for Human Genomics, Winston-Salem, NC 27157.

A combined linkage scan of 1,233 prostate cancer (PC) families was recently reported by the International Consortium for Prostate Cancer Genetics (ICPCG), with suggestive linkage findings for chromosomes 5q12, 8p21, 15q11, 17q21, and 22q12 (Xu et al. AJHG, 2005). However, PC is one of the most common cancers among men, with few men having overtly aggressive disease, which may lead to a large number of phenocopies, and which may explain why genetic linkage results of this disease have been difficult to replicate. To overcome this heterogeneity in disease etiology, a number of investigators have recently restricted genetic linkage analyses to the most severely affected men, but are limited by a small number of such families. Fortunately, the resources of the ICPCG are sufficiently large and broad to overcome small sample sizes. The ICPCG Clinical Committee defined aggressive PC as a series of or criteria: 1) regional or distant stage (based on pathology if available, otherwise clinical stage, T3, T4, N1, M1), or 2) Gleason grade score of 7 or higher, or 3) poorly differentiated grade (if no Gleason available), or 4) level of prostate specific antigen at diagnosis of 20 or higher, or 5) death from metastatic prostate cancer before age 65 years. We have recently completed a genome wide linkage scan of 189 families with three or more aggressive PC using both parametric heterogeneity (HLOD) and nonparametric allele sharing (Kong and Cox (KC-LOD)) methods, and find suggestive linkage results on chromosomes 5q (KC-LOD = 2.43), 6p (KC-LOD = 2.32), 11q (Recessive HLOD = 3.56), and 20q (Dominant HLOD = 2.50).

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Evidence for a prostate cancer predisposition locus on chromosome 22q in the Utah pedigrees. *N.J. Camp, J.M. Farnham, L.A. Cannon Albright.* Dept of Medical Informatics, Univ of Utah School of Medicine, Salt Lake City, UT.

We have pursued a hint of linkage for prostate cancer on chromosome 22, previously reported in a genome linkage scan in extended Utah pedigrees. A meta analysis of genome-wide screening data from the International Consortium for Prostate Cancer Genetics has reported significant evidence for linkage of this same region. The evidence for this region was contributed to, in part, by the Utah pedigrees, but included supporting evidence from other pedigree collections. Fifty-nine Utah pedigrees with at least 4 prostate cancer cases and no more than 2 meioses separating the prostate cancer cases were analyzed using the CIDR genomic search STRP marker set. We previously performed parametric linkage analyses using dominant and recessive models on four datasets resulting from a pedigree splitting algorithm. Three regions of interest (LODs > 1.9) were identified on chromosomes 1p, 5q, and 22q, with a maximum dominant TLOD of 2.09 at marker D22S685. Twelve of the Utah pedigrees had multipoint LODS > 0.588 ($p<0.05$) on chromosome 22 under the dominant model. Two previous studies reported hints of linkage within 15 cM of our peak. We have added fine mapping markers to a resolution of ~5cM on chromosome 22 in the 12 Utah pedigrees showing evidence of linkage. With this additional genotyping, 8 of these pedigrees maintain evidence of linkage (LOD > 0.588). Six flanking recombinants in these pedigrees, both telomeric and centromeric, strongly support an 8cM region, consistent with previous linkage reports for this chromosome.

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The search for cluster headache susceptibilty genes: genomewide scan and HCRTR2 candidate gene analysis. *L. Baumber[1], H. Harty[1], C. Sjostrand[2], J. Hillert[2], M. Leone[3], G. Bussone[3], M.B. Russell[4], R.C. Trembath[1].* 1) Dept Genetics, Univ Leicester, Leicester, United Kingdom; 2) Department of Neurology, Karolinska University Hospital, Sweden; 3) Carlo Besta National Neurological Institute, Milan, Italy; 4) Head and neck research group, Akershus University Hospital, Norway, and Faculty division Akershus University Hospital, University of Oslo, Norway.

Cluster headache (CH), a primary headache characterized by recurrent, unilateral, short-lived painful episodes, often with regular periodicity. CH prevalence has been estimated to be 1 per 500 of the adult population displaying a marked sex bias (female:male ratio 1:2.5-3.5). Whilst most frequently a sporadic disorder, twin concordance and familial studies support a genetic predisposition to CH. Segregation analysis favours an autosomal dominant disease trait. We have commenced a series of studies to elucidate the molecular genetic basis of predisposition to CH. We performed a polymorphic STR based genome-wide linkage analysis upon the five most informative multiplex families (ELOD 3.2) ascertained in Denmark. Despite no single chromosome generating a significant LOD score, evidence for suggestive linkage (NPL 2 or >) was observed for four putative disease loci. Investigation in additional families failed to confirm linkage for any nominal locus suggesting disease or locus heterogeneity in this disorder. We next assessed hypocretin receptor 2, a specific candidate gene recently implicated through a cohort association study. HCRTR2 encodes a G protein coupled receptor to orexin peptides, regulators of neuro-endocrine function. DNA sequence analysis of eight independent familial CH patients revealed no deleterious variants of HCRT2 in coding sequence and exon-intron junctions. Genotyping of two HCRTR2 SNPs in an extended cohort of European sporadic CH cases (n= 235) revealed no statistically significant difference in allele or genotype frequencies between cases and controls. These combined data emphasize the likely genetic complexity underlying CH and highlight the requirement for larger cohorts for replication of association studies in the condition.

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Linkage to 22q12 for a Nicotine Dependence Quantitative Trait in Australian and Finnish Samples. *S.F. Saccone[1], A. Agrawal[1], M.L. Pergadia[1], D.M. Dick[1], A. Todorov[1], A.-M. Loukola[4,2], U. Broms[2], H. Maunu[4,2], E. Widen[2], K. Heikkila[2], K. Morley[3], A. Baxter[3], M. Campbell[3], J.P. Rice[1], G.W. Montgomery[3], N.G. Martin[3], J. Kaprio[2], L. Peltonen[2,4], A.C. Heath[1], P.A.F. Madden[1].* 1) Washington University School of Medicine, St Louis, MO, USA; 2) University of Helsinki, Finland; 3) Queensland Institute of Medical Research, Australia; 4) National Public Health Institute, Helsinki, Finland.

Past research into the genetics of nicotine addiction has produced a variety of linkage signals with little concordance among results. This study used two samples, one Finnish and one Australian, where data collection and genotyping were completed separately in each country. For both samples, the genetic analysis of a quantitative trait, the maximum number of cigarettes smoked in a 24-hour period, found the maximum genome-wide evidence of linkage to occur at 22q12. The Australian sample consisted of 289 nuclear families containing probands for a heavy smoking phenotype which were genotyped at the Australian Genome Research Facility. In parallel, 155 nuclear families were identified in Finland and genotyped at the Finnish Genome Center. A regression of IBD sharing on the sum and difference of the trait values was performed using MERLIN-REGRESS. In each sample linkage peaks were detected at precisely the same location on chromosome 22. The maximum genome-wide multipoint LOD scores for the Australian and Finnish samples were 2.46 at 27 cM and 3.19 at 28 cM respectively. Combining the samples results in a multipoint LOD score of 5.16 at 27 cM. The marker D22S315 yields a singlepoint LOD score of 5.53 with an empirical p-value of 0.004 from 1000 singlepoint simulations. These results suggest genes involved in nicotine dependence lie in this region. One possible candidate is GRK3 (a.k.a. ADRBK2), the gene encoding the G protein receptor kinase 3, which has been shown to be associated with bipolar disorder. Build 35.1 of the NCBI physical map indicates D22S315 is intronic to GRK3. Funded by NIH grant DA12854, the Australian National Health and Medical Research Council, the European Union (QLG2-CT-2002-01254) and The Center of Excellence of the Academy of Finland.

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A Genome Scan for Retinal Pigmentary Abnormalities and Geographic Atrophy. *C.L. Thompson[1], G. Jun[1], B.E.K. Klein[2], R. Klein[2], J. Capriotti[1], K.E. Lee[2], S.K. Iyengar[1].* 1) Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI.

Genetic studies of age-related macular degeneration (AMD) often involve individuals with both choroidal neovascularization (CNV) and geographic atrophy (GA), but few genome-wide scans (GWS) have differentiated between these two distinct end points. In order to comprehend the genetics influencing pigmentary abnormalities (PA) and GA in AMD, we reanalyzed the data from our previous GWS on AMD (FARMS sample of 34 extended families including 346 sibling pairs) looking only at PA and ignoring CNV-related phenotypes. We also present a new GWS in the full Beaver Dam Eye Study (BDES) cohort (4,926 individuals and 1526 sibships), which includes data at baseline, 5 year and 10 year follow-up. Fundus photographs were taken for each subject and were graded on a new six-step retinal PA/GA scale ranging from absence to pure GA. Scores were adjusted for age. A model-free linkage analysis for PA/GA was performed on both samples for 338 markers covering all autosomes. Multipoint linkage analysis of the FARMS sample provided evidence for linkage with p-values < 0.01 in the 1q25, 5p13, 6q21-23 and 11q14 regions. The most significant peak was found on chromosome 1, in the region of Complement Factor H and Hemicentin-1 (empirical p-value of 4.38x10-3). Concordance with previous GWS for GA was found on 5p13. The loci on 1q25 and 6q21-23 have been observed in other GWS for AMD and may represent more general susceptibility genes. Linkage analysis in the BDES sample using the rate of change as a measure of progression replicated the peaks on chromosomes 5 and 6, suggesting that these loci may contribute to the rate of progression of pigmentary abnormalities toward GA. These findings suggest a complex, heterogeneous model for PA/GA.

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A high-density single-nucleotide-polymorphism (SNP) genomewide screen on extended autism families. *D.Q. Ma[1], J. Jaworski[1], I. Konidari[1], R.K. Abramson[2], H.H. Wright[2], M.L. Cuccaro[1], J.R. Gilbert[1], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) University of South Carolina School of Medicine, Columbia, SC; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Autism is a common neurodevelopmental disorder with a significant genetic component and locus heterogeneity. A genome-wide linkage analysis was performed on 26 extended autistic families (total 67 affecteds). Each family has 2 to 4 affecteds generating either avuncular or cousin pairs. For analysis, we used a high-density single-nucleotide-polymorphism (SNP) genotyping assay, the Affymetrix GeneChip Human Mapping 10K array. Two-point parametric linkage analysis demonstrates suggestive linkage evidence (HLOD>2.0) in regions at 1q23.3 (156-160 cM in the decode genetic map); 2q35-36.3 (210-230 cM); 5q23.2 (127 cM); 6p25.3 (16 cM); 11q14.1-21 (83-100 cM); 12q11-14 (56-80 cM); 12q22 (102 cM); and 14q11.2 (20 cM). Using a set of SNPs with R^2 <0.16 in the above peak region on chromosome 12, both multipoint parametric and non-parametric analysis displayed significant evidence for linkage in the region of 12q14.2 (76 cM to 81 cM) with the Max LOD score at 78 cM (HLOD=3.27 [REC]; LOD^*=3.59). Families with only affected males may represent a specific subgroup of autism. The linkage evidence on Chromosome 12 increased with a 2-point Max HLOD of 3.36 [REC] in the 17 extended families with only affected males. Multi-point non-parametric analysis supported this linkage with improved linkage evidence (Max LOD^2=3.93) between 76-78 cM. The novel linkage peak on chromosome 12 in this genome-wide screen further supports the hypothesis that there is substantial locus heterogeneity in autism and that extended families, along with gender effects, may help delineate these different loci.

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Genetic linkage analysis with affected relative pairs and their covariates: Application to prostate cancer linkage.

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Genetic linkage analysis of complex traits is complicated by heterogeneity of disease etiology, requiring statistical methods to account for non-genetic factors, or for gene-environment interaction, or perhaps for a primary gene when scanning for linkage with a secondary gene. To date, most linkage methods that account for covariates for affected relative pairs tend to be restricted to sib pairs, with the exception of the LODPAL method in S.A.G.E. This method is built on a conditional logistic model for the relative risk for different types of relatives (Goddard and Olson, AJHG, 2001). Although this method is appealing because it can be applied to arbitrary pedigrees, at times it can be difficult to maximize the likelihood due to model constraints, and it does not account for the dependence among the different types of relative pairs in a pedigree. To overcome these limitations, we have developed a new approach, based on score statistics for quasi-likelihoods. We have developed two types of tests: 1) a combined test for both linkage and heterogeneity, and 2) a test for heterogeneity, adjusted for linkage. Furthermore, we have developed robust methods that account for the dependence among different relative pairs within a pedigree. Fortunately, the methods can be formulated in terms of weighted least squares regression, with a special type of scaled covariate that accounts for the relationship of the members of a pair of relatives. One type of scaling leads to a first-order Taylor series approximation of the LODPAL method. Other types of scaling could be considered, implying that our proposed methods can be easily extended to improve power for different types of genetic mechanisms leading to disease. Application of our methods to prostate cancer linkage, and simulations, suggest that our approach is likely to be useful for a broad range of genetic linkage heterogeneity analyses.

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Linkage analysis of familial prostate cancer with recursive partitioning. *J. Sinnwell[1], D. Schaid[1], T. Therneau[1], W. Isaacs[2], International Contortium for Prostate Cancer Genetics.* 1) Dept Health Sci Res, Mayo Clinic Col Med, Rochester, MN; 2) Johns Hopkins University, School of Medicine, Baltimore, MD.

A family history of prostate cancer (PC) is one of the strongest risk factors for this disease, yet genetic linkage analysis of prostate cancer has been exceptionally challenging, with weak to moderate linkage signals and difficulty replicating linkage findings. To overcome these difficulties, the International Consortium for Prostate Cancer Genetics (ICPCG) has pooled 1,233 PC pedigrees with completed genome wide scans by microsatellite markers and with the following pedigree-specific covariates: mean age at diagnosis, number of men with PC, pattern of hereditary prostate cancer, and pattern of X-linked inheritance. To evaluate whether these covariates can be used to determine subsets of linked families, we implemented novel recursive partitioning (RP) methods for both parametric and model-free linkage analyses. The RP methods were implemented in the S-PLUS programming language, which grows and prunes a tree, with the leaves of a tree defined to be as homogeneous as possible. Here, we attempted to create leaves that are homogeneous with respect to linkage. The RP algorithm requires the following key measures which are applied recursively during the construction of a tree: 1) a goodness of split, with a larger value indicating a better way to split the data into subsets based on a covariate; and 2) a deviance, with a smaller value indicating a more homogeneous tree node. For the goodness and deviance measures, we used maximum heterogeneity lod scores for the parametric methods, and we used the Kong-Cox linear allele sharing method for the model-free approach. These methods were applied to the ICPCG data to determine homogeneous linkage subsets.

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Linkage analysis of familial prostate cancer with pedigree covariates by regression of nonparametric linkage scores. *K. Zarfas[1], D. Schaid[1], S. McDonnell[1], W. Isaacs[2], International Consortium for Prostate Cancer Genetics.* 1) Dept of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; 2) Johns Hopkins University, School of Medicine, Baltimore, MD.

Although prostate cancer (PC) has long been recognized as familial, with family history of this disease one of the strongest risk factors, genetic linkage analysis of prostate cancer has been exceptionally challenging. Because PC is one of the most common cancers among men, heterogeneity in disease etiology is likely a major reason why genetic linkage analyses have produced weak to moderate linkage signals, and replication has been difficult. To overcome these difficulties, the International Consortium for Prostate Cancer Genetics (ICPCG) has pooled 1,233 PC pedigrees with completed genome wide scans by microsatellite markers and with the following pedigree-specific covariates: mean age at diagnosis, number of men with PC, pattern of hereditary prostate cancer, and pattern of X-linked inheritance. To evaluate whether these covariates are associated with linkage to a chromosomal region, the pedigree-specific NPL scores calculated by Genehunter were regressed on the covariates by a series of linear regression models. A reduced model with an intercept-only was used to test for linkage; a full model with an intercept plus covariates was used as a combined test for both linkage and heterogeneity; and a test for heterogeneity, adjusted for linkage, was accomplished by contrasting the full versus reduced models. Permutations of the covariates were used to compute p-values for the combined and adjusted tests. Results from these analyses suggest interesting regions. The advantages of this approach are that it offers a mechanism to account for pedigree-specific covariates in pooled data, and provides rapidly computation of permutation p-values for both the combined test and the adjusted tests.

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Multifactorial genetic models for posterior subcapsular cataract using a genome scan. *G. Jun[1], B.E.K. Klein[3], R. Klein[3], K.E. Lee[3], S.K. Iyengar[1,2].* 1) Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, Ohio; 2) Ophthamology, Case Western Reserve University, Cleveland, Ohio; 3) Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, Wisconsin.

Posterior subcapsular cataract (PSC) is an age-related cataract whose etiology is incompletely understood and is influenced by environmental and genetic factors. We conducted a genome-wide scan (GWS) using 2252 individuals (N = 1015 sibpairs; 33 concordantly affected, ASPs; 160 discordantly affected, DSPs; and 822 concordantly unaffected sibpairs, USPs) from 487 pedigrees in the Beaver Dam Eye Study (BDES). The amount of PSC was graded within grid segments overlaid on a retro-illuminated photograph of the lens using standard protocols. We performed a model-free linkage analysis using PSC affection status (defined as 5 % of any grid segment). Of the most significant regions in the GWS, we assessed gene-gene and gene-environment interactions. We obtained evidence for linkage on 6q12 (D6S1053; $p=1.8x10^{-4}$), 9p21 (GATA187D09; $p=2.2x10^{-5}$), 12p11.23 (D12S1042; $p=1.2x10^{-6}$), 13q13.2 (D13S1439; $p=8.3x10^{-5}$), and 19q13.11 (D19S245; $p=3.8x10^{-5}$) in the GWS. These regions showed excessive mean allele sharing among ASPs and USPs, but decreased sharing among DSPs. The locus on 6q12 is shared with our previous GWS for cortical cataract. Different families were linked to 6q12 for PSC and cortical cataract. Linkage signals that were best explained by the interaction with heavy drinking and smoking were on 1q23 (main effect (ME): 0.28, interaction (I): $3x10^{-7}$) and 13q14 (ME: 0.52, I: $3.0x10^{-5}$), respectively. Significant gene-gene interaction with the marker D6S1053 was observed on 1q42 (ME: 0.84, I: $7x10^{-5}$), 9p21 (ME: 0.11, I: $1x10^{-4}$), and 10p11 (ME: 0.30; I: $1x10^{-4}$). In conclusion, genetic factors involved in PSC are multifactorial and shared with other cataracts.

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Genome-wide linkage screen of quantitative traits loci for autism. *C. Li[1,2], L. Jiang[1], G. Crockett[1], S. Anderson[1], J.L. Haines[1,3], J.S. Sutcliffe[1,3].* 1) Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Department of Biostatistics, Vanderbilt Univ, Nashville, TN; 3) Department of Molecular Physiology and Biophysics, Vanderbilt Univ, Nashville, TN.

Autism is a neurobehavioral spectrum of phenotypes characterized by deficits in the development of language and social relationships and by patterns of repetitive, rigid and compulsive behaviors. Twin and family studies have provided strong evidence for a genetic component in autism. Although many groups have performed genomic linkage screens using autism as a dichotomized trait, there havent been any systematic screening for autism-related quantitative traits. Using SOLAR, we performed a genome-wide linkage scan over the 22 autosomes on seven previously defined (Tadevosyan-Leyfer et al. 2003; Cuccaro et al 2003) autism-related quantitative traits in 356 combined Vanderbilt and AGRE (Autism Genetics Research Exchange) multiplex autism families. The seven quantitative traits are spoken language, social intent, compulsions and rigidity, developmental milestones, savant skills, sensory aversions, and insistence on sameness. The scores were transformed as necessary before analysis to conform to the normality assumption of the variance components method employed by SOLAR. We adjusted for the potentially different genetic effects between male and female subjects by including gender as a covariate in all analysis. In addition to gender, we also adjusted for other factors that may influence the outcome: age at interview for social intent and savant skills and birth order for spoken language. We carried out both two-point and multi-point analyses. Multi-point LOD scores of >3 were obtained for compulsions/rigidity and social intent on chromosomes 2q and 16p, respectively. All factors except insistence on sameness demonstrated LOD scores >2 on at least one chromosome. Results on 1q, 2q, 5q, 16p, and 17q overlapped with previous regions of interest based on linkage analyses of the dichotomous trait. These data support the utility in applying quantitative autism-related traits to the genome-wide search for susceptibility loci.

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High density whole-genome scan using single nucleotide polymorphisms for Fuchs Dystrophy. *Y.J. Li, N.A. Afshari, L. Zhang, E.R. Martin, S.G. Gregory, G.K. Klintworth.* Duke University Medical Center, Durham, NC.

Fuchs corneal dystrophy (FD) is a common late onset bilateral slowly progressive ocular disorder, which leads to a loss of corneal endothelium and development of guttae on Descemets membrane. It causes corneal edema and a severe impairment of visual acuity in the elderly population. The genetic basis of FD is not well studied. To date linkage screens have not been performed for FD. The only identified gene known to be responsible is COL8A2 on chromosome 1p, which was first found with a missense mutation in one early-onset FD family and one posterior polymorphous corneal dystrophy family. Further investigation showed associated mutations in late-onset FD. Analyses of COL8A2 in other cases of FD have been inconsistent. In order to identify the underlying genetic etiology of FD, we performed a genome-wide linkage scan using the Illumina linkage panel IV with 6144 SNPs markers on a set of 22 multiplex FD families. We used FASTLINK for parametric two-point linkage analysis with both recessive (REC) and dominant (DOM) affecteds only models and Allegro for multipoint analysis of interesting chromosomes (twopoint LOD scores > 1.5). We identified five regions with positive linkage signals from all analyses on chromosomes 1, 7, 15, 17, and X. The peak two point LOD score for the screen was found on chromosome 15q (LOD=3.26 for REC and 2.48 for DOM). Chromosome 17q (~107 to 126cM region) conatins our most overall significant results with LOD scores greater than 2 by both twopoint and multipoint analyses. The X chromosome result (LOD=1.80) is interesting given the preponderance of females with FD. Finally, the peak chromosome 1p region result (LOD >2) is located near the COL8A2 gene suggesting that at least a subset of families may have mutations in this gene warranting mutation screening of COL8A2 in these data. In summary, we report the first genome wide SNP screen in FD, provide evidence for the involvement of COL8A2 and identify novel regions for the location of FD risk genes.

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Fine mapping of the region on the chromosome 5q32 in Scandinavian families with celiac disease. *S. Adamovic[1], Å. Naluai[1,2], S. Nilsson[2,3], Å. Hellqvist[2], A.H. Gudjonsdottir[4], H. Ascher[4,5], J. Wahlström[1].* 1) Clinical genetics, Gothenburg, Sweden; 2) SWEGENE Genetics and Bioinformatics Core Facility, Göteborg, Sweden; 3) Chalmers University of Technology, Göteborg, Sweden; 4) Dept. of Pediatrics, The Queen Silvia Children's Hospital, Göteborg, Sweden; 5) Nordic School of Public Health, Göteborg, Sweden.

We performed a genome wide scan in 106 Scandinavian multiplex families with celiac disease (Naluai et al, EJHG 2001). After typing additional markers in the region on chromosome 5q31-33 the NPL score reached a significant level of 4,2. We continued our study by fine mapping a part of this region (approximately 6 cM) between markers D5S2017 and D5S434 in order to perform an association analysis. For this study we analysed 54 SNPs and 8 microsatellites. The SNPs were chosen with consideration to their validation status and position in the region. We focused on possible candidate genes, like genes involved in immunoreactions, cell cycling or apoptosis. SNPs chosen had minor allele frequencies above 0,1. The average distance between the markers was approximately 100-200 kb, except for two minor regions directly beneath the linkage peak where we increased the density of markers to 1 per 50kb. The analysis indicated some haplotypes that showed a nominal association ($p<0,001$). Although promising, it was not significant after correction and could not fully explain the linkage peak, so further investigation of the region is necessary.

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A genome-wide scan of Gilles de la Tourette syndrome. *D. Pauls[1], Tourette Syndrome Association International Consortium for Genetics[2].* 1) Dept Psychiatry/Neurodev Gen, Harvard Med Sch, Charlestown, MA; 2) Tourette Syndrome Association, Bayside, NY.

Twin and family study suggest that there is a significant genetic component important for the expression of Gilles de la Tourette Syndrome (GTS) and a number of linkage studies have reported suggestive evidence for linkage regions on 4q, 8p, and 11q. The present report describes the second genome-wide scan conducted by the Tourette Syndrome International Consortium for Genetics. A genome wide scan using approximately 400 microsatellite markers was completed on 327 affected sib-pairs and 20 multigenerational families. Altogether, over 2,200 individuals were genotyped in these families. Non-parametric linkage analyses were completed with GENEHUNTER and SIMWALK2. using both a diagnosis of GTS and/or chronic tics as affected. Six chromosomal regions with heterogeneity NPL scores over 3.1 were observed on 2p, 3p, 3q, 11q, 18p and 20p. The two regions with the highest NPL scores were 2p (NPL=4.17, $p < 0.000066$) and 20p (NPL=4.63, $p < 0.000023$). Fine mapping results in most of these regions are consistent with the initial findings. Of note is that the region on 11q is very close to the region reported in two previous studies. The results from this genome scan provide strong evidence for at least two and possibly three susceptibility loci for GTS. The finding on 11q is of particular import since it appears to be a replication of two earlier reports.

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Genome-wide scan for familial cutaneous malignant melanoma (CMM) susceptibility loci in a northeastern Italian population. *K.F. Kerstann[1], R. Steighner[2], M. Ter-Minassian[1], D. Calista[3], P. Minghetti[3], G. Landi[3], A.M. Goldstein[1], M.T. Landi[1].* 1) Genetic Epidemiology Branch, DCEG/NCI/NIH, Rockville, MD; 2) Core Genotyping Facility, Advanced Technology Center/NCI/NIH/DHHS, Gaithersburg, MD; 3) Dermatology Unit, Maurizio Bufalini Hospital, Cesena, Italy.

The incidence of CMM, the most severe cutaneous malignancy, has increased at a rate of 3-7% per year since the early 1960s in Western countries. Approximately 10% of all melanoma cases are familial. Several genetic susceptibility genes for CMM have been identified, including CDKN2A (9p21), CDK4 (12q14) -plus loci in the regions of 1p22 and 1p36, although the gene(s) within these regions have yet to be identified. These loci account for the genetic susceptibility in approximately 20-40% of familial CMM. Therefore, other genetic (and/or environmental) factors are likely involved in the majority of familial melanomas. To identify additional susceptibility loci for familial CMM we analyzed a racially homogeneous population from northeastern Italy. Sixty families with at least two cases of CMM were ascertained from the Bufalini Hospital in Cesena, Italy. Previous studies of Italian melanoma prone families found that approximately 33% of the families harbor CDKN2A mutations. However, only 7% (n=4) of our families had CDKN2A mutations. The families from Cesena were similar to other Italian families with CDKN2A mutations with regard to number of cases, pigmentation characteristics, and family members with multiple melanomas, dysplastic nevi or pancreatic cancer. Thus, we hypothesized that our families had an inherited susceptibility to CMM. We therefore, conducted a genome-wide scan of fifty-six families without mutations in any candidate gene for melanoma. We performed two-point linkage analyses based on a dominant model as well as multipoint and nonparametric analyses using 10cM microsatellite scan data. No evidence for linkage was observed for 12q14, 9p21, 1p22 or 1p36. Affecteds-only analyses identified three regions of interest (lod scores > 1.5), while pre-divided sample tests identified two additional regions (lod score >2). Follow-up analyses of these regions with increased marker density are underway.

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Bivariate genome-wide scan for phenotypes related to the metabolic syndrome in non-diabetic Chinese subjects from the Stanford Asia-Pacific program of hypertension and insulin resistance family study. *Y. Chiu[1], L. Chuang[2], A. Hsiung[1], The SAPPHIRe Study Group[3,4,5,6,7].* 1) Biostatistics & Bioinformatics, National Health Research Inst, Zhunan, Miaoli, Taiwan; 2) National Taiwan University, Taipei, Taiwan; 3) Division of Endocrinology and Metabolism, Tri-Service General Hospital, Taipei, Taiwan; 4) Faculty of Medicine, School of Medicine, National Yang-Ming University and Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; 5) the Executive Office, Taichung Veterans General Hospital, Taichung, Taiwan; 6) Department of Public Health Sciences and Epidemiology, School of Medicine, University of Hawaii and Pacific Health Research Insitute, Honolulu, Hawaii; 7) Department of Genetics, Stanford University, School of Medicine, Stanford, California.

Previously, we have identified regions with tentative evidence of linkage in the univariate genome-wide linkage scan for multiple correlated metabolic syndrome related traits. The common regions identified for the correlated traits might be due at least in part to the effects of pleiotropic genes. We therefore conducted bivariate linkage analyses for the correlated traits related to the metabolic syndrome (MS), aiming to dissect the genetic architecture affecting these traits. Results shown that the multiple traits analysis can improve the detection of quantitative-trait loci (QTLs), which have been found in the univariate linkage analysis. Further, QTLs whose effects were too small to be found in single-trait analyses were identified in the bivariate analyses.

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Gene mapping and evaluation of memory traits in the Caribbean Hispanics with familial Alzheimer disease. *J.H. Lee[1], H.-S. Lee[1], V. Santana[1], J. Williamson[1], R. Lantigua[1], M. Medrano[2], B. Tycko[1], E. Rogaeva[3], Y. Stern[1], P. St. George-Hyslop[3], R. Mayeux[1].* 1) Taub Inst & Sergievsky Ctr., Columbia Univ, New York, NY; 2) Universidad Tecnologica de Santiago, Santiago, DR; 3) Centre for Research in Neurodegenerative Diseases, Univ of Toronto, Toronto, Ontario, Canada.

Alzheimer disease (AD) comprise a complex array of subphenotypes. To simplify the AD phenotype and improve genotype-phenotype relation, we examined memory traits as endophenotypes because memory traits may represent biological traits underlying AD, and they have high to moderate heritability. Here, we report findings from a genome scan. We studied 1066 individuals from 210 Caribbean Hispanic families with late-onset AD. We evaluated both affected and unaffected family members for a battery of neuropsychological tests to measure memory, abstract reasoning, language and visuospatial functions. We conducted a variance component linkage analysis as implemented in SOLAR using 430 microsatellite markers. We adjusted for age, sex, and education in the polygenic model, and corrected for ascertainment. Because we found significant linkage in 10q, 12p, and 18q in our earlier AD genome scan, we concentrated on these regions. We observed several regions with LOD2, and to some extent, the findings differed by trait. Delayed recognition, a cognitively simple test, was a sensitive marker for familial AD where linkage peaks for the two traits often overlap. On the other hand, non-verbal recall test with low heritability yielded low LODs, and the pattern of linkage differed from other traits. For long-term recall and long-term storage, the pattern of linkage across the genome was similar. In 12p13 where we previously had a strong signal for AD, we observed only modest linkage (LOD=1.66) for delayed recognition but not for others. For 10q where others found strong linkage, we observed only a weak signal (LOD~1). Further, we observe novel chromosomal regions such as 18p11 (LOD=3.13) that were linked to memory traits, but not to AD. Here we show that we may be able to enhance power to map genes that contribute to AD using memory traits. We also show that we may be able to localize genes that are related to memory independent of AD.

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Genome-wide Scan for Schizophrenia in an Afrikaner Founder Population. *A. Woodroffe[1], J. Louw Roos[2], J.A. Gogos[3], G.R. Abecasis[1], M. Karayiorgou[4].* 1) Univeristy of Michigan, Department of Biostatistics, Ann Arbor, MI; 2) University of Pretoria & Weskoppies Hospital, Pretoria, RSA; 3) Columbia University Medical Center, Department of Physiology & Cellular Biophysics, New York, NY 10032; 4) Rockefeller University, Human Neurogenetics Laboratory, New York, NY 10021.

Schizophrenia is characterized by delusions, hallucinations, and disorganized speech or behavior. The disorder is very common, affecting 1 in every 100 people worldwide. Evidence for contribution from genetic factors include increased prevalence of schizophrenia in relatives of affected individuals. Twin studies indicate a 30-50% concordance rate for MZ twins and a 10-20% rate for DZ twins. For this study, we collected data from a sample of 481 Afrikaners. This is a founder population that has descended from about 2,000 Dutch founders. We carried out an extended, high-density genome-wide linkage scan using 2005 microsatellites at an average density of 2 cM. A maximum multipoint LOD score of 2.99 was observed at D13S1825 (133 cM), the most telomeric marker tested on chromosome 13q. The multipoint linkage peak extends from about 124 cM to the telomere. We also found a singlepoint LOD score of 2.92 at D13S285 (127 cM). These findings on 13q are consistent with the results of our previous lower density 10 cM scan which identified a multipoint LOD score of 2.23 at D13S285. The maximum singlepoint LOD score of 3.15 was observed at D1S2885 (45 cM). However, the observed multipoint LOD score at that marker was only 1.17. We have previously described a proband with paternal UPD of chromosome 1 in this population. We did not find any haplotype sharing between the UPD proband and other affected individuals at this marker density. Parent of origin linkage analysis to test for possible imprinting effects revealed suggestive evidence for maternal sharing on chromosome 1. In addition to the linkage analysis, we tested for association using the TDT. We did not find any disease associated alleles after correcting for multiple testing. We are currently employing large scale SNP genotyping to fine map candidate genes and regions on 13q and 1p.

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Bipolar Disorder with Psychotic Features shows Evidence of Linkage to Chromosomes 5,6,and 18. *B. Kerner, N.B. Freimer.* Ctr Neurobehavioral Genetics, Univ California, Los Angeles, Los Angeles, CA.

Purpose: The heterogeneity of psychiatric disorders is one of the major obstacles in the attempt to identify underlying genetic mutations. The presence of some phenotypic features that occur across diagnostic boundaries, such as psychosis, provides additional challenges. The familial aggregation of such features independent of existing diagnostic categories suggested the utility of such phenotypes in linkage analyses. We undertook a reanalysis of the NIMH Bipolar Genetics Initiative data sets Wave 1,3,and 4 using psychotic features to define affected status. **Methods:** 55 pedigrees in Wave 1 (484 individuals, 136 affected)and 187 pedigrees in Wave 3 and 4 combined (1170 individuals, 440 affected)were included in a whole genome scan using microsatellite markers. Pedigrees were selected for multiple members affected with psychotic symptoms. Relatives without a diagnosis of bipolar disorder were included as affected if they had a lifetime history of psychotic symptoms. The NPL option in MENDEL was used to calculate IBD sharing in relatives. **Results:** The most significant finding in Wave 1 was located on chromosome 5q34 between markers D5S820 and GABRA1 at 163 cM. The most significant finding in Wave 3 was located on chromosome 6q22 between markers D6S474 and D6S1040 and in Wave 4 on chromosome 18p11 and 18q12. Combined analysis of Wave 3 and 4 increased the signal on chromosome 18. When Bipolar Disorder was used as phenotype, only the signal on chromosome 6 in Wave 3 was evident. **Conclusion:** Bipolar Disorder with psychotic features appears to be a useful phenotype for linkage analysis. All loci reported here showed evidence for linkage in other genome scans for both bipolar disorder and schizophrenia. These loci may contain susceptibility genes for psychotic symptoms across diagnostic categories.

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Genome Screen for Atopic Dermatitis in the French EGEA families ascertained through asthmatic probands. *M. Dizier[1], M. Guilloud-Bataille[1], E. Bouzigon[2], C. Bétard[3], J. Bousquet[4], F. Gormand[6], J. Hochez[7], J. Just[8], A. Lemainque[3], N. Le Moual[9], R. Matran[1], F. Neukirch[1], E. Paty[1], I. Pin[1], D. Vervloet[5], F. Kauffmann[9], M. Lathrop[3], F. Demenais[2], I. Annesi-Maesano[9].* 1) INSERM U535, Villejuif, France; 2) INSERM EMI 00-06, Evry, France; 3) CNG, Evry, France; 4) INSERM U454, Montpellier, France; 5) Service de Pneumologie-allergologie, Hôpital Nord, Marseille, France; 6) Service de pneumologie, centre hospitalier Lyon-Sud, Pierre Benite, France; 7) INSERM U436, Paris, France; 8) Centre de diagnostic et traitement de l'asthme, Hôpital Trousseau, paris, France; 9) INSERM U472, Villejuif, France.

In the sample of 295 French EGEA families with at least one asthmatic subject, a genome screen was conducted to search for genetic factors specific to atopic dermatitis (AD) or shared by AD and asthma or by AD and allergic rhinitis (AR). The AD phenotype was based on self reporting AD status and age at onset. AR phenotype was based either on self reporting diagnosis or on symptoms while asthma phenotype was based on answers to a questionnaire plus the presence of bronchial hyper-responsiveness. The phenotype of non-specific organ allergic manifestations(NSOAM)defined by the presence of at least one of the allergic diseases (asthma, AR or AD) was also considered. Analyses were conducted using the Maximum Likelihood Binomial (MLB) method developed for linkage analysis in sibships. Evidence of linkage to the 11p14-q13 region was supported for AD (p=0.0001) as well as for NSOAM (p=0.0002) while linkage was only indicated to the 5q13 region for AD (p=0.002), when associated or not with asthma or AR, and to 5p13 (p=0.002) and 17q21 (p=0.003) for NSOAM. Detection of linkage to the 11p14-q13 region could be due to the presence of one or of more genetic factor(s), involved either specifically in AD or more generally in NSOAM. Further association studies with candidate genes in this region, may help to identify the underlying genetic mechanisms.

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Genomewide scan in Ashkenazi families shows linkage of ocular refraction to a quantitative trait locus on chromosome 1p36. *R. Wojciechowski[1,2], G. Ibay[1], C. Moy[3], D. Stambolian[3], J.E. Bailey-Wilson[1].* 1) IDRB, NHGRI, Baltimore, MD; 2) Epidemiology, Johns Hopkins U, Baltimore, MD; 3) Ophthalmology, U of Pennsylvania, Philadelphia, PA.

Purpose: The development of refractive error is mediated by both environmental and genetic factors. We performed regression-based quantitative trait locus (QTL) linkage analysis on Ashkenazi Jewish families to identify regions in the genome responsible for ocular refraction. Methods: We obtained refractive error measurements on individuals in 49 multi-generational American families of Ashkenazi Jewish descent. The average family size was 13.7 individuals and was composed of 3 generations. Recruitment criteria specified that each family contain at least 2 myopic members. The mean spherical equivalent refractive error in the sample population was -3.46D (SD=3.29) and 87% of individuals were myopic. Microsatellite genotyping with 387 polymorphic markers was performed on 410 people. We performed multipoint regression-based linkage analysis for ocular refraction using the statistical package Merlin-regress. To minimize the impact of extreme values on linkage statistics we also conducted analyses on a normalizing (log) transformation of refractive error. We estimated genomewide significance levels for ocular refraction and the transformed trait by generating random genotypes at each marker in 100 replicates of our pedigrees. We estimated empirical significance levels as the number of replicates in which the maximum LOD score exceeded that of the test sample for each trait. Results: Maximum LOD scores of 9.5 for ocular refraction and 8.7 for log-transformed refraction were observed at 49.1 cM on chromosome 1p36 between markers *D1S552* and *D1S1622*. The corresponding empirical genomewide significance levels were $p<0.07$ for ocular refraction and $p<0.01$ for log-transformed refraction, providing strong evidence for linkage of refractive error to this locus. The inter-marker region containing the peak spans 11 Mb and contains approximately 189 genes. Conclusion: We found genome-wide significant evidence for linkage of refractive error to a novel QTL on chromosome 1p36 in an Ashkenazi Jewish population.

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Type-1 Protein Phosphatase Inhibitor 4 (*I-4*) on Xp11.4-11.3 is Associated with Familial and Idiopathic Parkinsons Disease: The *Gene*PD Study. *G. Xu[1], J.B. Wilk[1], A.L. DeStefano[1], Q. Yang[2], J. Latourelle[1], S. Williamson[1], R. Prakash[1], J. Tobin[1], M. Sun[3], M.E. MacDonald[3], J.F. Gusella[3], R.H. Myers[1], for the GenePD investigators.* 1) Department of Neurology, Boston University School of Medicine, Boston, MA; 2) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 3) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Parkinson disease (PD), the second most common neurodegenerative disorder, is caused by degeneration of dopaminergic neurons in the substantia nigra and other areas of the brain. Mutations in five genes *parkin*, *-synuclein*, *DJ-1*, *PINK1*, and *LRRK2* have been identified to cause PD in a minority of families and more than ten PD linkage regions have been reported, with the susceptibility genes in these regions still under investigation. Three independent linkage analyses have provided compelling evidence for additional PD susceptibility genes on the X chromosome. We report modest evidence of linkage (multipoint LOD = 1.07) to PD affection status at 41Mb on the X chromosome in 283 affected sibling-pairs from the *Gene*PD Study. Thirty-seven SNPs, in or near possible PD candidate genes located from 40 to 47 Mb were evaluated for association to PD by chi-square tests and logistic regression in different genetic models. Association to PD affection was observed for c6231643 in two independent samples: Familial PD (p = 0.018) and Idiopathic PD (p = 0.016). C6231643 is 257 bp 3 of the type-1 protein phosphatase inhibitor 4 (*I-4*) gene. Haplotype analysis using three *I-4* SNPs (c2462683-c2462691-c6231643) also identified the evidence of association to PD in the two independent samples across genders. As a potentially important regulatory subunit of *PP1*, *I-4* may have impact on the phosphorylation processes triggered by dopamine, resulting in the development of PD.

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Model-independent linkage analysis and tests of association on regions of chromosomes 9 and 16 linked to idiopathic scoliosis. *C. Justice[1], B. Marosy[2], M.H. Roy-Gagnon[1], K.F. Doheny[3], E.W. Pugh[3], A.F. Wilson[1], N.H. Miller[2].* 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Department of Orthopaedic Surgery, JHU, Baltimore, MD; 3) CIDR, IGM, JHU SOM, Baltimore, MD.

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. It affects 2-3% of the pediatric population, and 0.2-0.5% of the population require active treatment. Idiopathic scoliosis is believed to be a complex genetic disorder in which expression of the disease state may depend on several genetic and possibly environmental factors. Previous studies have suggested autosomal dominant, X-linked and multifactorial modes of inheritance. As part of a large collaborative study of familial idiopathic scoliosis, 202 families with at least two individuals with a lateral curvature greater than or equal to 10 degrees were ascertained and clinically characterized. Phenotypes include degree of lateral curvature, curve type, age of onset and sex. A genomic-wide screen identified candidate regions on chromosomes 6, 9, 16 and 17 (Miller et al. 2005). The candidate regions on chromosomes 9 and 16 were genotyped with SNPs using the Ilumina platform. SNP marker density was ~ 58 Kb, with 519 SNPs genotyped on 9q31.3-q34.2 and 805 SNPs genotyped on 16p12.3-q22.2. Idiopathic scoliosis was analyzed both as a quantitative trait and as a qualitative trait, in which the curvature determining the threshold for affectation status was set at values of 10 and 30 degrees. Model independent sib-pair linkage analysis was performed on these various subsets. Tests of association using FBAT were performed with IS as a quantitative trait and as a qualitative trait. Haplotypes of two, three and four SNPs were also analyzed for association using FBAT.

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In search of the founder haplotype for the prostate cancer susceptibility locus on 7q11-21 in Jewish families using the Affymetrix 100K SNP chips. *D. Friedrichsen[1], J. Stanford[2], R. Wells[1], M. Janer[3], K. Deutsch[3], S. Kolb[2], H. Hung[1], P. Nelson[1], L. Hood[3], E. Ostrander[4].* 1) Dept Human Biol; 2) Public Health Sci, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Institute for Systems Biology, Seattle, WA; 4) Cancer Genetics Branch, NHGRI, National Institutes of Health, Bethesda, MD.

Hereditary prostate cancer (HPC) is a genetically heterogeneous disease with evidence for multiple loci contributing to overall susceptibility. One approach to reduce locus heterogeneity is to analyze HPC in Jewish families, which represent a more well-defined and genetically homogenous population. We previously reported a combined genome-wide scan of 36 Jewish HPC families where we identified a region of significant linkage on chromosome 7q11-21, with an empirical P value of 0.006. Our strategy for isolating this prostate cancer susceptibility gene is to identify the founder haplotype surrounding the founder mutation in at least a subset of these Jewish families. Previously reported founder haplotypes in Jewish families for susceptibility genes in other diseases have been 500 kb or larger. The Affymetrix 100K SNP chips have a SNP every 50 kb on average and chromosome 7 specifically has over 6,000 SNPs. The resolution of the 100K chips is potentially small enough to identify regions that may contain the founder haplotype. Currently, we are in the process of genotyping 90 members of the 18 Jewish PROGRESS families on the 100K SNP chips. This includes 51 affecteds and an additional 39 family members, which will be useful in determining chromosomal phase of the alleles. The data will be analyzed to determine regions where a specific haplotype pattern shared within families is also shared across several families for a large distance (greater than 500 kb) and where this pattern occurs more often in the shared affected haplotypes than in the other haplotypes in the families. After putative haplotypes are identified, SNPs from the HapMap project will be genotyped to confirm and further resolve the haplotypes identified. Finally, all exons in the remaining regions will be sequenced to discover the putative disease-associated mutation.

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Genome-wide association studies in founder populations: adding value by simultaneously testing for linkage using large families. *R.J. Thomson[1], J. Stankovich[2], S. Quinn[1], J. McKay[1,3], T. Albion[1], M. Bahlo[2], J. Silver[2], L. Fitzgerald[1], J. Dickinson[1].* 1) Menzies Research Institute, University of Tasmania, Hobart, Australia; 2) Walter and Eliza Hall Institute Of Medical Research, Melbourne, Australia; 3) International Agency for Research on Cancer, Lyon, France.

In terms of numbers of cases required, association studies are more efficient than linkage studies for detecting common alleles that confer susceptibility to disease. However linkage analysis with large pedigrees remains an efficient method to detect rare susceptibility variants. When genome-wide association studies are conducted in founder populations, it will often be possible to construct large pedigrees linking the cases. Thus the possibility arises of analysing such datasets to test for linkage as well as association.

In linkage analysis with large families it is impossible to analyse jointly all genotypes from all individuals using exact algorithms. However as marker density increases it becomes less crucial to analyse all individuals genotypes simultaneously. We propose a multipoint, pair-by-pair analysis of case genotypes using *Merlin 1.0*. This technique will be demonstrated, using *Hapmap* data to simulate genotypes in families, at the marker densities required for genome-wide association studies. Results for *Affymetrix* 10K and 50K SNP chips will be presented. Even at these lower marker densities, inference of haplotype sharing patterns is almost as accurate in a pair-by-pair analysis, as it is in an exact, whole-pedigree analysis.

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Genetic homogeneity in X-linked myopia locus at Xq28 (MYP1): Narrowing of the Critical Region and Exclusion of fifteen Known Genes Localized in the Interval. *S.K. Nath[1], U. Radhakrishna[2,3], R. Lyle[2], R. Raval[4], R. Singh[5], U.C. Patel[6], J.V. Solanki[6], V. Himabindu[7], S. Vishnupriya[7], V. Vittalrao[7], U. Ratnamala[3], S.E. Antonarakis[2].* 1) Arthritis & Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK; 2) Dept. of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 3) Green Cross Blood bank & Genetic Research Centre; 4) Dept. of Biochemistry, Gujarat Cancer and Res. Institute,; 5) Dr. P.L. Desai Eye Center,Ahmedabad; 6) Dept. of Animal Genetics & Breeding, Veterinary college, Anand,; 7) Dept. of Genetics, Osmania University, Hyderabad, India;.

Myopia is a common vision problem experienced by approximately one-third of the general population with a prevalence of 0.5-2.5% in Western Europe and US populations. The genes responsible for MYP1, MYP2, MYP3, MYP4, MYP5 and MYP6 have been mapped to chromosomes Xq28, 18p11.31, 12q21-q23, 7q36, 17q21-q22, and 22q12 respectively, but no specific gene mutations have yet been identified. We studied two large four-generation Indian pedigrees (UR006 & UR077) with isolated, non-syndromic myopia, with apparent X-linked inheritance (MYP1;MIM310460). The anomaly was only present in male members of the pedigree. The degree of myopia was variable ranged from -6 to -16.5 D with a mean of -13.33 D. In order to map the myopia locus in these families, we performed linkage analysis on 41 samples, 12 affected and 29 normals in both families, using polymorphic microsatellite markers covering the entire X chromosome. Marker DXYS154 which is located in pseudoautosomal (PAR) region in distal Xq28, showed no recombination with the phenotype with a combined maximum LOD score of 4.42 at theta = 0 under an autosomal recessive model. Other markers in the Xq28 region that showed no recombination with the phenotype included DXS1108, DXS8087 and F8i13. Observation of recombination in family UR006 refined the disease locus to 1.2 cM region flanked by markers DXS1073 and DXYS154. Mutations in the CTAG1B, CTAG2, GAB3, MPP1, F8Bver, FUNDC2, VBP1, RAB39B, CLIC2, TMLHE and (PAR) HSRY3, SYBL1, IL9R, SPRY3, CXYorf1 were excluded by direct sequence analysis.

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Evidence of a Novel Postaxial Polydactyly Type B Locus in a Costarrican Family. *J. Peralta[1,2], P. León[1], H. Raventós[1], L. Almasy[2].* 1) Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San José, Costa Rica; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Postaxial Polydactyly (PAP) is a congenital hand/foot malformation characterized by the presence of an extra ulnar/fibular digit. In PAP Type A (PAP-A) the digit is functional and articulates with the fifth metacarpal/metatarsal, but in Type B (PAP-B) it is present as an skin appendix without bone tissue. Both types segregate as autosomal dominant traits with incomplete penetrance, although there have been reports of recessive inheritance of PAP-B. Genetic and allelic heterogeneity are common features of PAP. Several loci have been linked with either PAP-A or the mixed type PAP-A/B. Allelic variants of one of those loci, *GLI3*, are responsible for PAP-A, PAP-A/B, Preaxial Polydactyly and Greig Cephalopolysndactyly. Our goal was to garther evidence regarding the involvement of known PAP loci in the development of PAP-B. Given the known genetic heterogeneity of PAP, we concentrated our analysis in one large family from Costa Rica (F01) that has affected individuals in all four generations and segregates only PAP-B as a dominant trait. *GLI3* (7p13), *PAP-A/B* (7q22), *PAP-A2* (13q21-q32) and *PAP-A3* (19p13.2-p13.1) as well as key genes involved in limb development, *IHH*, *SHH*, *dHAND*, and *HOXD13*, were selected as possible candidates. A total of 50 STRs located in the candidate gene regions, the HOXA, B, C and D gene clusters, and inside the ninth intron of GLI3, were genotyped in 17 of the 20 affected family members. Using an affected-only analysis and a disease allele frequency of 0.001, linkage was excluded (LOD-2) for all candidate loci. For the *GLI3* intragenic locus Z=-4.79@=0. Recently a balanced translocation t(4;7)(p15.2;q35) has been described in association with PAP-B [Galjaard, 2003]. STRs near the reported translocation breakpoints genotyped in F01 showed no evidence of linkage with PAP-B. Our results suggest the existence of a novel PAP locus responsible for the isolated PAP-B phenotype observed in F01. The calculated power of F01 (3expected LOD7) should be enough to localize this novel locus.

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Fine mapping of candidate regions in African Americans with sarcoidosis: Confirmation of linkage to chromosome 5p15.2. *R. Sinha[1], S.K. Iyengar[1], B.A. Rybicki[2], M.C. Iannuzzi[3], R.C. Elston[1], C. Gray-McGuire[1].* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, MI; 3) Division of Pulmonary, Critical Care, and Sleep Medicine, Mt. Sinai School of Medicine, New York, NY.

Sarcoidosis, a systemic granulomatous disease of unknown etiology, likely results from an environmental effect on a genetically susceptible individual. This disease is much more prevalent in women than in men and, in the United States, African Americans are both more commonly and more severely affected than Caucasians. This study is a follow-up of the recently published first genome scan for sarcoidosis susceptibility genes conducted in an African American sample comprising 229 African American nuclear families ascertained through two or more sibs with a history of sarcoidosis (Ianuzzi et al. Genes and Immunity, 2005). Areas of interest from the genome scan include chromosomes 5p15-13, 5q11, 5q35, 2p25, 3p25, 3q12, 9q34, 11p15 and 20q13. These fine mapping regions were analyzed using both the original Haseman-Elston regression (dependent variable is the squared sib-pair trait difference) and the weighted Haseman-Elston regression (dependent variable is a weighted average of the squared sib-pair trait difference and the squared mean-corrected sum) with two new improvements. The first improvement was inclusion of the available half sib pairs in the regression, which increased our sample size by 20%, and the second improvement was estimation of the mean correction term from the data. The marker with the strongest signal, D5S1486 on 5p15.2, had an initial p-value of 0.007, which decreased to 10^{-6} with the inclusion of half sibs. These results demonstrate the presence of a susceptibility gene on 5p15.2 as well as the utility of correctly including half sibs in the analysis.

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Exclusion of candidate genes for a hereditary type of telangiectasia. *M. Amyere[1], L. Boon[2], MB. Mulliken[3], M. Vikkula[1].* 1) Lab Human Molec Genetics, Christian de Duve Inst, Brussels, Belgium; 2) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium; 3) Center for Vascular Anomalies, the Childrens Hospital and Harvard Medical School, Boston, MA, USA.

Telangiectases are abnormal dilatation of end vessels, mainly venules, but occasionally involving capillaries and arterioles in the subpapillary plexus in the upper dermis. The Hereditary haemorrhagic telangiectasia (HHT or Rendu-Osler-Weber disease) is characterized by epistaxis, mucocutaneous telangiectases and visceral arteriovenous malformations (AVMs) caused by mutations in Endoglin (ENG) or activin receptor-like kinase-1 (ALK1, ACVRL1). Hereditary benign telangiectasia (HBT) is another, rare variant. Affected individuals present with cutaneous, punctate, radiating or arborising telangiectases. HBT is distinguished from HHT, by the lack of AVMs and absence of mucosal lesions. These two telangiectasias are inherited as an autosomal-dominant disorder with variable penetrance. Another similar hereditary disorder, CM-AVM, is caused by mutations in RASA1. These patients have more homogeneous, round-to-oval pinkish-red cutaneous lesions, atypical capillary malformations, which are sometimes associated with AVMs. We report a family with cutaneous telangiectasias with an autosomal dominant pattern of inheritance. The affected individuals develop large, heteregenous telangiectasias that are not associated with AVMs. This condition could be a more extensive variant of HBT. We performed a genome-wide linkage analysis using microsatellite markers and SNP genotyping assay with the GeneChip Human Mapping 10K Array. The analysis excludes the three candidate genes, endoglin, ALK-1 and RASA1. This provides evidence for existence of a different causative gene for this type of hereditary telangiectasia. (http://www.icp.ucl.ac.be/vikkula); (vikkula@bchm.ucl.ac.be).

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Genome wide linkage analysis of a novel hereditary progressive hyperpigmentation disorder. *T. Vogt[1], M. Amyere[2], M. vikkula[2].* 1) Dept Dermatology, Univ Regensburg, Regensburg, Germany; 2) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université Catholique de Louvain, Brussels, Belgium.

Familial progressive hypermelanosis is a new variant of a hereditary pigmentation disorders without associated symptoms. This phenotype has so far been observed only in five families, all living in close proximity in a small town in south-east of Germany (Zanardo et al., 2004). The phenotype, with an autosomal dominant inheritance with reduced penetrance, consists of progressive diffuse, partly blotchy hyperpigmentation, multiple café-au-lait spots, intermingled with scattered hypopigmented appearing maculae, and lentigenes. Histological and ultrastructural section from the hyperpigmented spots display strong basal hyperpigmentation of the epidermis with numerous melanophages containing large amounts of pigment. In contrast, the hypopigmented appearing macula show a slight basal hyperpigmentation of epidermis, but virtually no melanophages in the upper dermis. The restricted area of occurrence of the disease and the extreme rarity of the phenotype suggest a common origin and thus a founder effect for this genetic defect. Based on this hypothesis, we performed a genome-wide linkage analysis in the five families using the GeneChip Human Mapping 10K Array SNP genotyping. The results demonstrate the presence of significant linkage peaks with maximal LOD of 2.28 assuming the smallest genetic distance between the five families, on the long arm of chromosome 12. All affected individuals shared the same haplotype in this locus. This finding suggests that a gene involved in melanin distribution is located on chromosome 12. (http://www.icp.ucl.ac.be/vikkula); (vikkula@bchm.ucl.ac.be).

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Linkage analysis of ocular refraction as a quantitative trait in an adult population. *A.P. Klein[1,2], P. Duggal[3], K. Lee[4], R. Klein[4], J.E. Bailey-Wilson[3], B.E.K. Klein[4].* 1) Departments of Oncology and Pathology Johns Hopkins School of Medicine, Baltimore MD; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore MD; 3) Statistical Genetics Section, NIH/NHGRI/IDRB, Baltimore, MD; 4) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison WI.

Ocular refraction refers to the power of the external lens to bring images into focus on the retina. Refractive errors, myopia (nearsightedness) and hyperopia (farsightedness) are common conditions that require corrective lenses. It has been well established that myopia clusters within families and linkage to regions on chromosome 12q, 17q, 18q and 22q has been demonstrated through studying highly myopic individuals. The potential for genetic effects through the entire range of refraction has been less well studied. In a study of 221 dizygotic twin pair, regions on chromosomes 3q, 4q, 8p and 11p have been reported to be linked to refraction. Using data collected as part of the Beaver Dam Eye Study, we previously demonstrated substantial positive correlation for refraction as a quantitative trait between siblings (0.39), parents and offspring (0.26) after adjustment for age, sex. In order to localize the genes influencing refraction in this population non-parametric genome-wide linkage analyses was conducted on 881 sibling pairs within 487 extended pedigrees (2231 genotyped individuals) using 385 autosomal microsatellite markers. Sibling pair linkage analysis (singlepoint and multipoint) using SIBPAL (S.A.G.E version 4.5) was performed on quantitative refraction, and refraction adjusted for age, education and nuclear sclerosis. Refraction was adjusted for nuclear sclerosis because nuclear lens opacity has been shown to decrease refraction (more myopic). Both asymptotic and empirical p values using Monte Carlo permutations were obtained. Three novel regions meet the Lander-Kruglyak criterion for suggestive evidence for linkage ($p<0.00074$). Additionally, our results replicated two of the previously reported linkage regions. Linkage analysis of the extended pedigree data will also be presented.

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Linkage Analysis for Laterality Genes in Pedigrees Ascertained for Autism. *A. Lu[1], S.J. Spence[2], N. Kono[1], R.M. Cantor[1], D.H. Geschwind[2].* 1) Dept Human Genetics, David Geffen School of Medicine, Univ of California, Los Angeles, CA; 2) Dept of Neurology, David Geffen School of Medicine, Univ of California, Los Angeles, CA.

Human brain asymmetry for language is strongly associated with handedness, and typical patterns of brain and behavioral asymmetry are reported to be disrupted in a number of neurodevelopmental disorders. For example, an excess of non-right-handed individuals has been observed among a sample of 78 children with Autism (Gillberg, 1983). We observed the same pattern in a sample of 275 children with Autism and 53 of their unaffected siblings, from the Autism Genetic Research Exchange (AGRE), a cohort of approximately 500 nuclear families ascertained for at least two children with Autism Spectrum Disorder. A GEE logistic regression model, used to account for the dependence among siblings, revealed an increased frequency of 24% left-handed or ambidextrous individuals versus 4% right-handed, ($p<0.006$). We reasoned that non-right-handedness could serve as an Autism endophenotype, and whole genome multipoint linkage analysis was conducted in the AGRE sub-sample of 91 genotyped pedigrees containing 420 individuals and at least one non right-handed member, including parents. Handedness was modeled as the result of a single recessive gene with a frequency of 0.45 and 50% penetrance, consistent with the 10% population prevalence, and the analyses were conducted using the parametric option of the GENEHUNTER software. Only 2 regions had a lod score greater than 1.0 in this preliminary study: 8q24.21 at 140 cM with a lod score of 2.4 and 20q13.12 at 62 cM with a lod score of 1.3. These findings, while not significant at the genome-wide level, warrant additional analyses in independent samples of a larger size.

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Chromosome 10 in Late-onset Alzheimer Disease. *X. Liang[1], B. Anderson[1], N. Schnetz-Boutaud[1], J. Bartlett[1], B. Lynch[1], P.C. Gaskell[1], H. Gwirtsman[1], L. McFarland[1], M.L. Bembe[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].*
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Late-onset Alzheimer disease (LOAD) is a complex neurodegenerative disorder resulting from multiple genetic and nongenetic factors. Several genome screens have consistently suggested that chromosome 10 harbors at least one locus for LOAD. However, the region on chromosome 10 is still large. To narrow down the peak region and make association analysis more feasible, we analyzed our large family-based dataset using both covariate and subset analyses to increase the homogeneity.

36 SNPs evenly spaced across 80.2 Mb region on chromosome 10 were genotyped in 725 multiplex families (1204 affected sib pairs). We analyzed the data using Ordered Subset Analysis (OSA), and a two point LOD score of 2.50 was obtained at marker rs1319013, p=0.002 in 45% families when the families were ordered from low to high by ApoE LOD score. Marker rs14327 generated two point LOD score of 1.94 in 13% families when the families were ordered from low to high using covariate of the linkage signal on chromosome 12 (p=0.009). In the autopsy confirmed subset, rs10826594 generated the peak of LOD=2.07 in OSA multipoint analysis when the covariate ApoE LOD score ranked families from low to high. In the ApoE all4 subset (all AD patients have at least one ApoE 4 allele), marker rs3127242 generated two point LOD of 1.40 when families were ordered by linkage signal on chromosome 12 from high to low (p=0.04). In the ApoE non4 subset (none of AD patients has ApoE 4 allele), marker rs3750686 generated parametric two point LOD score of 1.54. This marker also had a two point LOD of 2.30 in OSA using the linkage signal on chromosome 12 from low to high as a covariate.

Our data suggest that there is a locus associated with LOAD in the region near rs1319013 (62 cM) and rs3750686 (103 cM) on chromosome 10. These two regions most likely contain gene(s) related to late-onset Alzheimer Disease.

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Identification of granuloma-loci through lead-overlap in 100K genome wide association experiments in Sarcoidosis and Crohn Disease. *J. Hampe[1], A. Franke[1], C. Becker[2], R. Valentonyte[1], M. Krawczak[3], J. Müller-Quernheim[4], M. Schürmann[5], S. Nikolaus[1], T. Kühbacher[1], P. Nürnberg[2], S. Schreiber[1].* 1) Institute of Clinical Molecular Biology, Christian Albrechts University, Kiel, Germany; 2) Cologne Center for Genomics, University of Cologne, Germany; 3) Institute of Medical Statistics and Biometry, Christian Albrechts University, Kiel, Germany; 4) Department of Pneumology, University Hospital Freiburg, Germany; 5) Institute of Human Genetics, University of Lübeck, Germany.

Crohn disease (CD) and Sarcoidosis share granulomas as a pathognomonic histological feature of tissue inflammation and do cluster clinically. The spectrum of involved organs and the disease course are quite different on the other hand. The joint analysis of the genetic risk profile gives the chance to investigate common etiological pathways possibly specific for the overlapping granuloma trait. The age- and sex-matched samples from 393 CD patients, 400 sarcoidosis patients and 399 control individuals were genotyped on the 100K Affymetrix GeneChip system. Single point genotypic and allelic association tests were performed for markers fulfilling the following criteria in controls: i) no evidence of departure from Hardy-Weinberg-Equilibrium at the p=0.05 level, ii) minor allele frequency >1% and iii) Call rate >95%. Out of the 83,268 SNPs meeting these criteria, 2466 SNPs showed significant association at the p=0.05 level in CD and 3135 in sarcoidosis. 364 markers overlapped between the two analyses, which is significantly more ($p<0.001$) than expected by random selection alone. Similar results were obtained when choosing a significance threshold of $p<0.001$ for the association signals to compare. A total of 34 SNPs for CD (including *CARD15*) and 132 for sarcoidosis fulfilled this more stringent criterion with an overlap of 3 SNPs on chromosomes 11, 13, and 19 between both diseases, which is again much more than expected by chance. We conclude that the overlapping association leads might represent common susceptibility variants predisposing to granulomatous inflammation while other loci and environmental factors lead to the different tissue distribution in the two disorders.

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Cytogenetic Characterization and Molecular Association of Patients with the Silver - Rusell Syndrome (SRS). *G. Vasquez[1], C. Muneton[1], A.H. Campo[2], A. Ruiz-Linares[3], H. Bedoya[2], F. Quintero-Rivera[4], J.L. Ramirez-Castro[1].* 1) Dept Genetics, Antioquia Univ, Medellin, Colombia; 2) Laboratorio de Genética Molecular, Sede de investigación Universitaria (SIU), Universidad de Antioquia. Med., Colombia; 3) The Galton Laboratory, Department of Biology (Wolfson House), University College London, Stephenson Way, London, UK; 4) Center for Human Genetic Research, MGH, Boston, MA.

Silver-Russell syndrome (SRS) is a heterogeneous genetic disorder mainly characterized by several clinical findings, in which patients show prenatal and postnatal growth retardation. SRS is genetically sporadic, but several reported cases demonstrate dominant or recessive patterns of inheritance, suggesting a genetic cause. On the other hand, maternal uniparental 7 chromosome disomy, and maternal imprinting have shown to play an important role in some SRS patients. In the present study we found a SRS affected child in each of seventeen studied families. All the patients and their families were from Antioquian population (Colombia). All of them had a normal karyotype. For the microsatellites markers assays, the D7S2422 and D7S813 markers for the 7p11.2-p13 chromosome showed a biparental segregation. For the Hardy-Weinberg equilibrium test, the same markers were in equilibrium in the population (parents and patients). The Linkage Disequilibrium and Association Tests (TDT) for the markers D7S2422 and D7S813 applied first to parents and them to the whole population of parents and children showed that no association exist among these markers and the GRB10 related SRS gene. In conclusion, the transmission analysis of haplotypes and respective genealogies suggests an autosomal recessive type of inheritance; in which affected SRS descendant and normal parents are present.

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Following up genome scans for plasma apolipoprotein E (apoE): analysis of chromosome 17q positional candidate genes in GENOA families. *M.S. Leduc[1], L.C. Shimmin[1], K.L.E. Klos[1], C.F. Sing[2], E. Boerwinkle[1], J.E. Hixson[1].* 1) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Plasma apoE plays a central role in metabolism of several lipoproteins that are important CVD risk factors (triglycerides, LDL, HDL). The common apoE isoforms only explain about one-fourth of the genetic variation in apoE levels. To identify other genes that influence apoE levels, we performed a genome-wide search in families from the GENOA study (Genetic Epidemiology Network of Atherosclerosis). To increase genetic and environmental homogeneity, we performed linkage scans in families stratified by ethnicity, lipoprotein levels, and BMI. We found significant evidence for linkage on chromosome 17q in Hispanic families for two strata including low cholesterol (LOD=3.32 at 103cM) and low cholesterol-high HDL (LOD=3.20 at 110cM). This linkage signal was replicated in African-American families, with evidence for linkage on 17q for 2 strata including low cholesterol-high HDL (LOD=1.76 at 73cM) and low BMI-low TG (LOD=1.89 at 85cM). In this study, we are following-up these 17q linkage signals using a positional candidate gene approach. We identified 10 candidate genes that are located in the linked region with known roles in metabolic pathways of lipoproteins or other CVD risk factors. We selected 32 SNPs for genotyping in the GENOA families. We used FBAT to find associations between plasma apoE levels and the common ApoH isoforms in Caucasians (APOH*H1, p=0.02) and Hispanics (APOH*H3w, p=0.02), but in unstratified families. We also found associations for ACE1 in unstratified Hispanics (p=0.02), as well as in Hispanics stratified by low cholesterol (p=0.02). In addition to these single SNP analyses, we are directly determining multi-SNP haplotypes using hybrid cell lines that are monosomic for chromosome 17. These molecular haplotypes will greatly facilitate accurate haplotype estimation in the GENOA families for multi-SNP association studies, as well as conditional linkage analyses to ascertain whether associated haplotypes are responsible for the original linkage peak on chromosome 17q.

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Linkage to GABR cluster with exclusion of GABRG2 and GABRA1 genes in a large Colombian family with GEFS+. *N. Pineda-Trujillo[1], J. Carrizosa[2], R. Mehon[1], W. Arias[3], C. Alzate[3], G. Bedoya[3], W. Cornejo[2], A. Ruiz-Linares[1,3].* 1) University College London, London-United Kingdom; 2) Servicio de Neurologia Infantil, Facultad de Medicina, Universidad de Antioquia Medellin-Colombia; 3) Grupo de Genética Molecular, Universidad de Antioquia, Medellín-Colombia.

Generalized epilepsy with febrile seizures plus (GEFS+) is a clinical and genetically heterogeneous disorder. Four genes (SCN1B, SCN1A, SCN2A and GABRG2) and four loci (FEB1-FEB4) have been associated with the disorder. However, the exclusion of these GEFS+ candidate genes/loci in large families has suggested that other genes may play a role in the aethiology of this disorder. Our purpose was to evaluate candidate GEFS+ genes/loci in a large Colombian family with the disorder, by typing tightly linked polymorphic microsatellites. Results indicated linkage to GABR cluster (GABRG2, GABRA1, GABRA6 and GABRB2). However, sequencing of the GABRG2 coding region resulted in failure of identifying a mutation in this gene. Typing additional polymorphic markers in the GABR cluster revealed a recombinant individual for marker SHGC-132760, located in between GABRG2 and GABRA1, confirming exclusion of GABRG2 as responsible for the disorder in this family. Furthermore, a clear disease segregating haplotype was identified. GABRA1, which previously was associated to Juvenile myoclonic epilepsy was also excluded by sequencing its coding region. Sequencing of the GABRB2 and GABRA6 genes, the two remaining genes in this cluster, is underway. Our results suggest that high likely either GABRB2 or GABRA6 will present a mutation responsible for GEFS+ in our family, which would provide evidence of a second g-aminobutiric acid receptor associated with GEFS+. N. P-T. is funded by a COLCIENCIAS studentship.

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Sex matters: an example of differential oligogenetic control of induced oncogenesis in male and female rats. *C. Fischer[1], B. Koelsch[2], A. Kindler-Rohrborn[2].* 1) Human Genetics, University of Heidelberg, Heidelberg, Germany; 2) Department of Neuropathology, University of Bonn, Medical Center, Bonn, Germany.

The inbred BD rat strains provide a suitable model system for the genetic dissection of susceptibility and resistance to the development of nervous system tumors, as they exhibit a broad spectrum of reactivity to tumor induction by the alkylating carcinogen N-ethyl-N-nitrosourea (EtNU). Among these BDIX and BDIV rats are highly susceptible and completely resistant, respectively, to the development of peripheral nervous system (PNS) tumors, (malignant schwannomas) which arise predominantly in the trigeminal nerves. We previously reported on the genetic mapping of 7 loci controlling tumor incidence and/or survival time in segregating (BDIX x BDIV) crosses on chromosomes 10 (Mss1) chromosomes 1 (Mss2), 3 (Mss3), 6 (Mss4), 13 (Mss5) and 15 (Mss6) as well as 10 (Mss7, centromeric portion). Interestingly, most of these loci mediate allele- and sex-specific effects of variable strength ranging from minor influences on tumor development to complete tumor resistance. This is reflected by the fact that male (BDIX x BDIV) F2 rats exhibit a two fold higher incidence of EtNU-induced schwannomas than females as well as a shorter survival time. The sex effect is even more striking when analysing gene-gene interactions. We found numerous interactions of which the most striking one was in female animals between two completely independent loci that displayed also main effects. For interaction analysis the false discovery rate to control statistical errors has been used. A number of human tumors too arise with a marked sex bias. Our study serves as a model for diseases with sex-specific oligogenetic susceptibility mechanisms. Grant sponsor: Krebsforschung International, e.V., Düsseldorf, Germany; Dr. Mildred Scheel Stiftung für Krebsforschung (Grant 10-1830 Ki 1), Bonn, Germany; Bonfor Program of the Medical School, University of Bonn, Germany.

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Fine mapping in nuclear families using linkage and linkage disequilibrium (LD) information: Dissection of linkage signals according to parental genotype. *J. Wicks.* ARC Ctr Complex Systems, Univ QLD, St Lucia, Australia.

Gene mapping studies use linkage and LD information to try to locate genetic variants which influence a phenotype. Defining and distinguishing between linkage and LD information has been a focus of research for some years, as scientists attempt to locate variants showing the strongest LD signal, and thereby determine causal variation, or variation in perfect LD with causal variation. Without functional studies of some kind, gene mapping studies cannot distinguish between variation which is directly causal and that in perfect LD with a causal variant. This defines the limit of what is possible with gene mapping studies, and also sets the ultimate aim of association mapping - which is to locate causal variants and those in perfect LD with causal variants. A major challenge, then, is to determine as much as possible about the hidden pattern of LD between an *observed* variant associated with a phenotype, and an *unobserved* causal variant responsible for the association. Addressing this question in the case of nuclear family genetic data, and with sibling phenotypic data for a qualitative or quantitative trait, it can be shown that LD and linkage information can be dissected by considering the linkage signals from the subsets of the data defined by the different possible genotypes in the parents. By subsetting the data in this way, very distinctive patterns emerge which provide a basis for distinguishing between *perfect* LD ($r^2 = 1$, $|D'| = 1$), *complete but imperfect* LD ($r^2 < 1$, $|D'| = 1$), and *incomplete* LD ($r^2 < 1$, $|D'| < 1$) (Wicks et al. 2005). Using these ideas and borrowing from the ingenious Homozygous Parents Affected Sib Pair and Marker Association Segregation Chi-Squares methods, as well as numerous other authors, two novel methods emerge. One is a parametric method of analysis of the underlying LD pattern, and the other is a very simple and elegant non-parametric method for choosing the most likely variant for causality, or for perfect LD with a causal variant, from a collection of genotyped variants in and around a gene. Application of the methods to simulated data sets shows that they provide useful results when compared to other LD mapping methods.

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Interaction between AGT and ACE in Alzheimer disease. *P.G. Bronson[1], N.N. Wall[1], K. Welsh-Bohmer[2], J.L. Haines[3], J.R. Gilbert[1,2], M.A. Pericak-Vance[1,2], E.R. Martin[1,2].* 1) Center for Human Genetics, Duke Univ Med Center, Durham, NC; 2) Dept of Medicine, Duke Univ Med Center, Durham, NC; 3) Center for Human Genetics Research, Vanderbilt Univ Med Center, Nashville, TN.

We previously examined the effect of the intron 16 insertion/deletion and 7 single nucleotide polymorphisms (SNPs) in the angiotensin-converting enzyme gene (ACE) on chromosome 17. Several markers were significantly associated with AD, but it is unclear which contribute to risk due to strong linkage disequilibrium (LD) between markers. ACE is part of the renin-angiotensin system (RAS), which regulates blood pressure; hypertension is a risk factor for AD (Amouyel et al. 2000). Angiotensinogen, encoded by the AGT gene on chromosome 1, is part of the RAS pathway. Because of the biological interaction between ACE and AGT (Bis et al. 2003), we examined effects of 5 tagging SNPs in and around AGT. The SNPs were genotyped in a family dataset (371 families with 1,234 phenotypically discordant sibling pairs) and an independent case-control dataset (595 cases and 967 controls). We did not find significant association in the family dataset using the pedigree disequilibrium test (PDT) and the genotype-PDT. However, we did find significant association in the independent case-control dataset. The SNP1 G allele had an odds ratio (OR) of 1.34 (95% CI, 1.02 to 1.74; p=0.033). This association remained when we adjusted for age at exam, sex and number of APOE4 alleles (OR=1.42; 95% CI, 1.03 to 1.95; p=0.033). Carriers of the SNP1 AG genotype were at greater risk than carriers of the AA genotype in the crude (OR=1.39; 95% CI, 1.06 to 1.82; p=0.019) and adjusted (OR=1.45; 95% CI, 1.05 to 2.01; p=0.025) genotypic models. We found significant interaction between AGT and ACE in our crude and adjusted logistic regression models, with p-values ranging from 0.010 to 0.040. Although several AGT and ACE markers showed significant main and interactive effects on AD, different levels of significance in different analyses may reflect LD with an undiscovered functional variant. Further study is necessary to understand the interaction of AGT with ACE in AD.

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Association between Alzheimer disease and Nicastrin. *N.N. Wall[1], P.G. Bronson[1], K. Welsh-Bohmer[2], J.L. Haines[3], J.R. Gilbert[1,2], M.A. Pericak-Vance[1,2], E.R. Martin[1,2].* 1) Center for Human Genetics, Duke Univ Med Center, Durham, NC; 2) Dept of Medicine, Duke University Medical Center, Durham, NC; 3) Center for Human Genetics Research, Vanderbilt Univ Med Center, Nashville, TN.

Several studies have found an association between the Nicastrin gene (NCSTN) on chromosome 1 and Alzheimer's disease (AD). Nicastrin interacts with presenilins 1 & 2 in the -secretase complex involved in proteolysis of -amyloid precursor protein (-APP). The lysis of APP causes the formation of amyloid plaques, a hallmark of AD pathology. We examined the effect of 5 intronic single nucleotide polymorphisms (SNPs) in the NCSTN gene on AD. SNPs were genotyped in a large AD family dataset (371 families with 1,234 phenotypically discordant sibling pairs) and an independent case-control dataset (595 cases and 967 controls). We did not find any significant allelic association in the family dataset using the pedigree disequilibrium test (PDT). However, we found significant genotypic association in one of the SNPs using the genotype-PDT. Specifically for SNP3, the G allele trended toward significance (p=0.052) and the GG genotype was significantly associated with AD (p=0.031). We found significant allelic and genotypic association with one of the SNPs in the independent case-control dataset. The SNP5 G allele had an odds ratio (OR) of 1.37 (95% CI, 1.11 to 1.69; p=0.003). This association remained when we adjusted for age at exam, sex and number of APOE4 alleles (OR=1.31; 95% CI, 1.03 to 1.68; p=0.03). Carriers of the SNP5 GG and AG genotype were also at increased risk in crude and adjusted linear (AA vs. AG vs. GG) and genotypic (GA vs. AA and GG vs. AA) models (p-values between 0.002 and 0.044). We found no linkage disequilibrium (LD) (r^2 =0.01) between SNP3 and SNP5 in the family or independent case-control dataset. Since both SNP3 and SNP5 are intronic with no known functional significance, it is likely that the association with AD is due to yet undiscovered functional variant(s) in LD with these SNPs. Further study is necessary to understand the genetic involvement of specific polymorphisms of NCSTN as well as the biological mechanism affecting AD risk.

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No evidence of interaction between chromosomes 1q31 and 10q26 in age-related maculopathy (ARM). *Y.P. Conley[1, 2], J. Jakobsdottir[3], D.E. Weeks[2, 3], T.S. Mah[4], R.E. Ferrell[2], M.B. Gorin[2, 4].* 1) Department of Health Promotion & Development, University of Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, PA; 3) Department of Biostatistics, University of Pittsburgh, PA; 4) Department of Ophthalmology, University of Pittsburgh, PA.

We recently discovered susceptibility genes for ARM on chromosome 10q26 of similar importance to ARM risk as the Complement Factor H (CFH)gene on 1q31 that has been replicated by several groups. We performed high density SNP genotyping on 1q31 and 10q26 and found very strong associations of the CFH and PLEKHA1/LOC387715/PRSS11 loci with ARM; we also detected a weaker signal over the GRK5/RGS10 loci. We employed the genotype-IBD sharing test as well as logistic regression to test for an interaction between SNPs from CFH and each of the genes on 10q26. We found no significant interaction between the two chromosomal regions. We tested several models: the baseline model including only the mean, three additive models which assumed an additive effect at first, second or both loci, three dominant models which incorporated dominant effects and three interaction models of additive/additive, additive/dominant and dominant/dominant interaction. Using the Akaike information criteria we compared the models; in the case of PLEKHA1, LOC387715 and PRSS11 an additive model including CFH was the best fitted model but when considering GRK5/RGS10 and CFH, an additive model including CFH only was the best fitted model. These results suggest that the effects of PLEKAH1/LOC387715 and CFH act independently towards ARM susceptibility, irrespective of the form of endstage ARM disease, and the possible effects of GRK5/RGS10 on ARM risk are very weak at best. We have found no interaction between CFH, PLEKHA1, LOC387715 or PRSS11 with self-reported smoking information, but our preliminary results suggest that there might be an interaction between GRK5/RGS10 and smoking in ARM. We are continuing to investigate these potential interactions.

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Comprehensive Association analysis of genetic polymorphisms in the cell division cycle 2 (CDC2) gene with late-onset Alzheimer's disease. *J. Bartlett[1], X. Liang[1], N. Schnetz-Boutaud[1], B. Anderson[1], B. Lynch[1], P.C. Gaskell[2], H. Gwirtsman[1], L. McFarland[1], M.L. Bembe[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Alzheimer disease is a progressive complex neurodegenerative disease, characterized by neurofibrillary tangles in the neurons of the cerebral cortex and hippocampus and the deposition of amyloid within senile plaques and cerebral blood vessels. Several genome scans have indicated that chromosome 10 has at least one locus for this disease. Recent evidence has shown linkage between Alzheimers disease and two loci on chromosome 10 (marker D10s1255 and D10S583). Our association analysis focused on the cell division 2 gene (CDC2) which is close to one of the linkage regions (2Mb from D10S1225) and has previously been associated with risk in AD with an odds ratio of 1.78. Biologically, CDC2 is thought to be one of the main candidate kinases involved in PHF-tau formation because it can phosphorylate normal tau in vitro, compromising the ability of tau to stimulate microtubule assembly in AD. Five single nucleotide polymorphisms spanning the entire gene were selected and examined for association for late onset AD. A family-based dataset including 1337 discordant sibpairs and an independent dataset of 745 cases and 998 controls were collected in a Caucasian population in which the affected individuals were diagnosed at an age greater than or equal to 60 years of age. These datasets have >80% power to detect an odds ratio of 1.38. Neither dataset demonstrated any association with late-onset AD in our samples with all p-values >0.16. We conclude that the contribution of common genetic variants in CDC2 to the risk of developing AD is likely to be non-existent or very low.

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Patterns of linkage disequilibrium are consistent within and between ethnic groups: sampling vs. ancestry in the HapMap project data. *F.C.L. Hyland, C.R. Scafe, H. Isaac, F.M. de la Vega.* Applied Biosystems, 850 Lincoln Centre Dr., Foster City, CA 94404, USA.

The recent release of the HapMap data raises important questions about the extent to which similarities and differences observed in the patterns of sequence variation between populations are an effect of sampling rather than due to intrinsic differences; and whether results from the Yoruban African population are predictive for African Americans. We computed metric linkage disequilibrium (LD) maps (expressed in LD units, or LDUs) for 104,824 SNPs on chromosomes 6, 21 and 22 in four HapMap populations. We compared these to 28,155 SNPs previously genotyped using TaqMan SNP Genotyping Assays on samples of 45 unrelated individuals from comparable Caucasian, Chinese and Japanese populations, and from African Americans. 12,206 SNPs were in common across the two data sets. The correlation of minor allele frequency (MAF) between two independent samples within an ethnic group is much higher than correlation between ethnic groups. The correlation between MAF in African Americans and Yorubans is similar to that within any other ethnic group. In contrast, LDU patterns are highly consistent between as well as within ethnic groups. The mean correlation between LDU across ethnic groups is 0.9993 (SD 0.0006). The mean correlation between LDU within samples of an ethnic group is 0.9992. This is consistent with the hypothesis that LD is largely driven by recombination rate; hence the fixed locations of steps and plateaux in the LD map. Correlation between LD across ethnic groups is 10 times higher than the correlation between LDU and physical locations (mean 0.9935, SD 0.0016), and highly significant. The length and LDU locations of the African American and Yoruban LD maps are consistent. These results confirm that differences in MAF or patterns of LD between ethnic groups observed in the HapMap data reflect underlying differences rather than sampling effects, and that the patterns inherent in the LD maps are largely consistent within and between ethnic groups. This raises the possibility of developing a standard LD map for association mapping in all populations if suitably scaled.

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A Genome-wide Search for Susceptibility Genes for Intraocular Pressure: The Africa America Diabetes Mellitus (AADM) Study. *C. Rotimi[1], G. Chen[1], A. Adeyemo[1,2], K. Agyenim-Boateng[3], B. Eghan[3], J. Acheampong[3], A. Doumatey[1], J. Zhou[1], O. Fasanmade[4], T. Johnson[4], F. Akinsola[4], T. Rufus[4], G. Okafor[5], J. Oli[5], F. Ezepue[5], A. Amoah[6], S. Owosu[6], S. Akafo[6], Y. Chen[1], F. Collins[7].* 1) Natl Human Genome Ctr, Howard Univ, Washington, DC; 2) University College Hospital Department of Pediatrics and Chemical Pathology, Ibadan, Nigeria; 3) University of Science and Technology Department of Medicine, Kumasi, Ghana; 4) University of Lagos, College of Medicine, Endocrine and Metabolic Unit, Lagos, Nigeria; 5) University of Nigeria Teaching Hospital, Department of Medicine and Ophthalmology, Enugu, Nigeria; 6) University of Ghana Medical School Department of Medicine and Surgery, Accra, Ghana; 7) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Glaucoma is one of the leading causes of blindness worldwide. High intraocular pressure (IOP) is the major risk factor for glaucoma. Populations of recent African origin including West Africans and African-Americans have demonstrated increased risk for glaucoma. A genomic scan was conducted to identify loci linked to IOP in 246 type 2 diabetes sib-pairs from two West African ancestral populations of African-Americans. A total of 390 microsatellite markers were scored for an average spacing of 9 cM with no gaps greater than 20 cM. Multipoint linkage analyses were performed using the SOLAR package. Evidences of significant linkage was observed on chromosomes 5q22 with a LOD score of 3.4 (p=0.000038). Suggestive linkage was also observed on chromosome 14q22 with a LOD of 2.5 (p=0.000348). The strong signal on chromosome 5 is reinforcing previous studies and provides additional evidences that chromosome 5 may indeed contain susceptibility loci for glaucoma in multiple human populations. The evidence provided in this study is particularly important given the evolutionary history of these West African populations and the recent ancestral relationship to African Americans; a population with one of the highest rates of diabetes and associated complications including glaucoma in the world.

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Genome-Wide Scan for Schizophrenia in the Molecular Genetics of Schizophrenia (MGS1) Collaboration Pedigrees Suggests Linkage in 8p23.3-p12 and 11p11.2-q22.3. Results of Fine Mapping. *P.V. Gejman[1], B.J. Mowry[2], R. Freedman[3], D.F. Levinson[4], F. Amin[5], J.M. Silverman[6], C.R. Cloninger[7], D.W. Black[8], W.F. Byerley[9], R.R. Crowe[8], A.L. Hinrichs[7], A.R. Sanders[1], C.H. Jin[7], J. Duan[1], C. Hou[1], N.G. Buccola[4], N. Hale[8], A. Olincy[3], J.P. Rice[7], B.K. Suarez[7].* 1) ENH & Northwestern Univ, Evanston, IL; 2) Univ Queensland, Queensland, Australia; 3) Univ Colorado Health Sciences Center, Denver, CO; 4) Univ Pennsylvania, Philadelphia, PA; 5) Emory Univ, Atlanta, GA; 6) Mount Sinai School of Medicine, NY, NY; 7) Washington Univ, St. Louis, MO; 8) Univ Iowa, Iowa City, IA; 9) Univ California, San Francisco, CA.

We report the clinical characteristics of a schizophrenia sample of 413 pedigrees, 266 of European Ancestry (EA) and 147 of African-American (AA) ancestry together with the results of a genome scan (STRP interval: 9 cM) and of follow-up fine-mapping. A family was required to have a sibling proband with schizophrenia (SZ), plus one or more additional siblings with SZ or schizoaffective disorder. Linkage analyses included 357 independent full-sibling ASPs (256 EA; 101 AA) and 101 all possible half-sibling ASPs (15 EA; 86 AA). Non-parametric multipoint linkage analysis of all families detected two regions with suggestive evidence for linkage on 8p23.3-p12 and 11p11.2-q22.3 (empirical Zlr ? 2.65), and a third region on 4p16.1-p15.32 in AA families in an exploratory analysis. The most significant linkage peak was in 8p; its signal was mainly driven by the EA families. Zlr scores >2.0 in 8p were observed from 29.4 cM to 60.4 cM (CIDR map). The maximum evidence was a Zlr = 3.35 (equivalent Kong-Cox LOD = 2.43) at D8S1771 (50 cM); there appear to be two peaks, both telomeric to NRG1. There is a paracentric inversion common in Europeans within this region whose effect on the linkage evidence remains unknown in this and in other previously analyzed samples. We have performed SNP based fine-mapping of chromosome 8p and of several other regions. Fine mapping of 8p has not significantly altered the significance or length of the peak. Fine mapping data for other chromosomal regions is under analysis.

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Evidence for linkage of serum alkaline phosphatase levels: a genome-wide scan in the Framingham Offspring Study. *J-P. Lin[1], A. Cupples[2], C. O'Donnell[3].* 1) Office of Biostatistics Research, National Heart, Lung and Blood Institute, Bethesda, MD; 2) Dept. of Biostatistics, Boston University School of Public Health, Boston, MI; 3) Framingham Heart Study, National Heart, Lung and Blood Institute, Framingham, MI.

The serum activity of alkaline phosphatase (ALP), produced mainly in the liver, bone, kidney, intestine and placenta, correlates with bone formation rates and bone densities. Twin studies have shown that serum ALP variation is largely determined by genetic factors with heritability estimates ranging from 40-80%. A genome scan on serum ALP activity in mice revealed three major quantitative trait loci (QTL) on chromosomes other than those where the genetic loci known to be involved in the production of ALP isoforms are located. To date, no linkage analysis on serum ALP levels in humans has been reported. We carried out a 10 cM genome-wide scan in a community-based Caucasian cohort, the Framingham Heart Study. ALP was measured in the first examination of the offspring cohort (mean age = 36 years). Our study population consisted of 330 families with 1255 individuals being both genotyped and phenotyped, including 1322 full sibling pairs, 52 half sibling pairs, 665 cousin pairs, and 89 avuncular pairs. Using variance-component linkage methods implemented in SOLAR, the heritability was estimated as 39%; after age, sex, serum bilirubin, serum albumin and total serum protein adjustment. The genome-wide linkage analysis demonstrated evidence of linkage of ALP levels to chromosome 11p with a LOD score of 2.9 at 22 cM and to chromosome 1q with a LOD score of 2.0 at 233 cM. Human ALP isoforms have been previously mapped to chromosomes 1p36-34 and 2q34-37. Our study suggests that there may be QTLs that control serum ALP levels residing on chromosomes 11p and 1q, in regions that differ from those containing the genes encoding ALP isoforms.

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A QTL on 12q Influencing a Marker of Inflammation and Body Mass Index in Women: NHLBI Family Heart Study. *J. Wu[1], J.S. Pankow[2], R. Tracy[3], K.E. North[4], L. Djoussé[5], R.H. Myers[5], M.A. Province[1], I.B. Borecki[1].* 1) Division of Biostat, Washington Univ Schl Med, St. Louis, MO; 2) Div Epidemiol Community Health, Univ MN; 3) Univ VT; 4) Dept Epidemiol, Univ NC; 5) Boston Univ Schl Med.

It has been recognized that obese individuals are intrinsically in a state of chronic inflammation, as indicated by positive correlations between serum levels of the inflammation marker C-reaction protein (CRP) and various anthropometric measures of obesity. To explore the hypothesis that a gene(s) may underlie this relationship, we conducted bivariate linkage analyses of body mass index (BMI) and CRP in Caucasian families of the NHLBI Family Heart Study. A set of 404 highly polymorphic markers with map density of ~10 cM were genotyped by the NHLBI Mammalian Genotyping Service (Marshfield, WI). Genotyping in African-American families is underway. CRP was log transformed to normalize its distribution, and both CRP and BMI were adjusted for age, age^2, age^3, sex, and field center. Variance components linkage analysis as implemented in SOLAR was performed in the combined data (1982 subjects) and within gender (892 men and 1092 women). CRP exhibited significant genetic correlations with BMI in women ($_G$=0.59, p<0.001) and the combined sample ($_G$=0.40, p<0.001), but not in men ($_G$=0.19, p=0.39). We detected a maximum bivariate LOD score of 3.64 on chromosome 12q24.2-24.3 (at 138 cM) in women. Within this region exists an interesting candidate gene, AMPK-BETA1 (AMP-activated protein kinase -1), whose product is a regulatory subunit of AMPK. Activation of hypothalamic AMPK was shown to block leptin's effects on food intake and body weight. Recent findings also suggest that AMPK activation could influence CRP production by modulating adipocyte secretion of interleukin-6. A suggestive linkage signal (lod=2.83) was found in the combined sample on chromosome 8q22.3 (118 cM). The highest peak (lod=1.96) obtained in men was localized on chromosome 16p13.3-p12 (20 cM). The above bivariate peaks were substantially higher than their respective univariate lods at the same locus for each trait. In summary, our results indicate that chromosom12q24.2-24.3 may harbor a QTL jointly regulating BMI and CRP in women.

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Localization of candidate regions for a novel gene for situs inversus/Kartagener syndrome. *I. Gutierrez-Roelens[1], Th. Sluysmans[2], M. Amyere[1], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian De Duve Institute of Cellular Pathology, Brussels, Belgium; 2) Division of Pediatric Cardiology, Cliniques universitaires St Luc, University of Louvain Medical School, Brussels, Belgium.

Asymmetric positioning of internal organs is characteristic to vertebrates. The normal left-right anatomic positioning, situs solitus, sometimes does not occur normaly leading to laterality defects, situs inversus, consisting of a complete inversion of the global left-right axis. Studies in animal models have shown that laterality decisions are mediated by a cascade of genes that lead to asymmetric expression of Nodal, LEFTA, LEFTB and PITX2 in the lateral plate mesoderm. Search for mutations in genes implicated in left-right patterning in animal models allowed to identify genes associated with heterotaxia defects in man. However, these genes explain only a small percentage of human situs defects suggesting that other genes must play a role. In this study, we report a consanguineous family composed of two unaffected parents and three children, two of which presented situs inversus with or without Kartagener syndrome. On the basis of the family history, we hypothezise autosomal recessive mode of inheritance. Genotype analysis with polymorphic markers did not show linkage with the known genes or loci causing laterality disorders. Array CGH did not detect a duplication or a microdeletion to be the cause of the affection either. Thus, genome wide screening using 10K Affymetrix SNP chips was performed allowing the identification of two regions of autozygosity, one in chromosome 1 and the other on chromosome 7. In the chromosome 1 locus, the strong candidate gene encoding the kinesin associated protein 3 (KIF3AP) was not mutated based on SSCP/heteroduplex analysis. The data give the basis for the identification of a novel situs determining gene, which is also implicated in the Kartagener syndrome. (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.be/vikkula).

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An Application of the IBS-IBD Method to the Genetic Analysis of Alcoholism. *Q. Yue[1], V. Apprey[1], R. Taylor[2], G.E. Bonney[1].* 1) National Human Genome Ctr, Howard Univ, 2216 6 Street Room 206 NW Washington, DC 20059; 2) Department of Pharmacology, Howard Univ, Washington, DC 20059.

Our IBS-IBD transformation method uses the probabilistic relationship between identical by state and identical by descent sharing in sib-pairs to calculate the test for linkage. This method was particularly designed for sib-pair data without parental genotypes. In this paper, we applied this method to the Genetic Analysis Workshop (GAW) 14 COGA microsatellite data on alcoholism. We find that the markers D2S1329, D2S1333, D6S495, D7S1790, D7S820, D7S1799, D11S4464, D17S975, D21S1440 and D22S1267 have LOD scores of 5.96, 2.11, 2.93, 2.01, 2.25, 2.06, 2.79, 3.16, 2.71 and 3.43 respectively. Some of the markers listed above were also reported by other researchers, for example, the marker D7S820 was also reported by Nsengimana et al (GWA 14, 2004) with a LOD score of 2.04. Interestingly, the regions franked the markers D21S1440 and D6S495, were reported by Zhang et al (GWA 14, 2004) using SNPs, not microsatellite markers. However, the marker D2S1329 with the highest LOD score of 5.96 was not reported by any of the papers in GWA 14 meeting. In conclusion, using our IBS-IBD method on sib-pairs with microsatellite markers without parental genotypes, one can still find most of the linkage regions found by using sibling and parental genotypes or even SNPs data, and one may also find some additional interesting regions that may be caused by the population hypotheses in our IBS-IBD method.

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High density genome scanning using Affymetrix chips: Indications of genome coverage and capture of variation based on the HapMap ENCODE regions. *M.R. Barnes[1], T.K. Bhinder[1], R. Elango[1], N. Goodgame[1], M.G. Ehm[2], S. Chissoe[2], E. Lai[2].* 1) Molecular Genetic Informatics, GlaxoSmithKline, Harlow, Essex, United Kingdom; 2) Discovery and Pipeline Genetics,GlaxoSmithkline, 5 Moore Drive, Research Triangle Park, North Carolina, 27709.

High density genome wide association studies have for many years been a long-term technological and analytical goal for human genetics. The new generation of SNP-based oligonucleotide arrays offers one technical solution to achieving this goal. In this study we evaluated the gene coverage and capture of variation by the commercially available, 117,000 SNP, Mapping 100K array from Affymetrix and the early access version of the soon to be commercially available, 528,000 SNP, 500K Mendel array. We have summarized the information captured by the SNPs in these maps using the ten HapMap-ENCODE regions as a model to estimate genome-wide map performance. We stratified measures of map performance in several ways, firstly in terms of gene coverage, secondly in terms of overall capture of information on variation, thirdly in terms of capture across the spectrum of SNP allele frequencies, and finally in terms of capture of potentially functional variation. The results of these analyses suggest that these technologies may indeed offer effective genome coverage at a cost that decreases substantially with map scale. However some of these benefits come at a price: as map scale increases many more markers are used than are needed to adequately scan the genome based on the criteria evaluated. This results in unnecessary resources and time being spent on the storage, analysis, and interpretation of these markers. Further still this will give rise to an increase in nominally significant associations. A very large proportion of these associations are likely to be false. This emphatically shifts the challenge of whole genome association studies from the technological to the analytical domain.

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Genomewide linkage scan for intraocular pressure among Mongolian families shows evidence of linkage on chromosome 7p. *H. Kim[1], J. Kim[2], S. Cho[3], M. Lee[4], S. Jung[1], J. Seo[4,5].* 1) Biochemistry, Ewha Womans University, Seoul, Korea; 2) Dept Biochem, Coll Medicine, Hallym Univ, Chuncheon, Korea; 3) Graduate School Public Health, Seoul National Univ, Seoul, Korea; 4) Ilcheon Instit Molecular Medicine, Seoul National Univ,Seoul, Korea; 5) Dept Biochem, Coll Medicine, Seoul National Univ, Seoul, Korea.

High intraocular pressure (IOP) is the strongest known contributing factor causing glaucoma, a group of diseases in which retinal ganglion cell death and optic nerve degeneration lead to blindness. IOP is under both genetic and environmental influences. With age it is known that the IOP increases among western population, while that decreases in orient. It suggests that the loci regulating the IOP might be different to the glaucoma loci known so far. IOPs were measured by air tonometer for 475 individuals (37 Mongolian families, the average family number is 13), most of them are Khalkh tribes living Orhontol Som in Selenge Imac, Mongolia. The genotyping was performed using the ABI PRISM linkage mapping Sets MD-10. Initial analysis was carried out for the 189 genotyped individuals with the largest family numbers. The Pedigree Relationship Statistical Test (PREST) was used to identify pedigrees. The initial nonparametric multipoint analysis gave evidence of linkage on chromosomes 4p, 7p, and 15q with LOD scores exceeded 2.5. Maximum multipoint LOD score of 3.2 was observed at marker D7S519. These results suggest that a locus on chromosome 7p12 may contain a gene with a large effect on IOP in this popuation. Identification of a susceptibility locus in this region may eventually lead to a better understanding of gene-environment interactions in the regulation of IOP.

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Segregation analyses and genome linkage scan of Bone Mineral Density in a European sample of families selected through a male osteoporotic subject. *C. Pelat[1], I. Van Pottelbergh[2], M. Cohen-Solal[3], J.M. Kaufman[2], M.C. de Vernejoul[3], M. Martinez[1,4].* 1) INSERM EMI00-06, Evry , France; 2) Endocrine department Gent University, Belgium; 3) INSERM U349, Paris, France; 4) Northwestern University, Evanston, IL.

Bone Mineral Density is one of the most important clinical predictors of osteoporotic fracture risk. BMD is a high heritable trait but the genes responsible are incompletely defined. The aim of this study was to assess the genetic determinism of BMD, as determined by segregation analyses under the regressive models. In addition, we performed a genome-wide linkage scan in order to identify chromosomal regions influencing these phenotypes. We have ascertained a sample of 100 extended pedigrees originating from France and Belgium. Families were selected through a male relative with osteoporosis (Zscore<-2 either at the Lumbar Spine or the Femoral Neck). Epidemiological data and BMD measures were collected for all living relatives accepting to participate in the study. Prior to segregation and linkage analyses, we constructed trait-residuals to adjust for the effects of age, sex and BMI. The best fitting segregation models included the effects of both a major gene and residual family correlations. Major gene effects were found more significant for BMD-FN than for BMD-LS. In addition, these analyses identified highly significant gene-environment interactions for BMD-FN (gene-age) and for BMD-LS (gene-age and gene-BMI). The genome-wide scan was performed using 458 microsatellites markers in our family data (85 families informative for linkage; 430, 321 and 325 sib, cousins and avuncular pairs respectively). We used model-free linkage analyses based on the variance component approach, as implemented in the computer program Merlin. Linkage analyses revealed a number of regions linked to either BMD-LS or BMD-FN. The positive regions did not overlap much across the two phenotypes. Altogether, the stronger signals were observed on chromosomes 11, 13, 17 and 22 (Lod=4.3, P=10-5; Lod=2.05, P=0.001; Lod=3.15, P=0.00007; Lod=2.72, P=0.0002).

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Combined Analysis from Eleven Linkage Studies of Bipolar Disorder Provides Strong Evidence for Susceptibility Loci on Chromosomes 6q and 8q. *M.B. McQueen[1], N.M. Laird[2], Genetic Determinants of Bipolar Disorder Working Group.* 1) Epidemiology, Harvard School of Public Health, Boston, MA; 2) Biostatistics, Harvard School of Public Health, Boston, MA.

Several independent studies and meta-analyses aimed at identifying genomic regions linked to bipolar disorder have failed to find clear and consistent evidence for linkage regions. Our hypothesis is that combining the original genotype data provides benefits of increased power and control over sources of heterogeneity that outweigh the difficultly and potential pitfalls in the implementation. We conducted a combined analysis using the original genotype data from eleven bipolar disorder genomewide linkage scans comprised of 5179 individuals from 1067 families. Heterogeneity among studies was minimized in our analyses by using uniform methods of analysis and a common, standardized marker map, and was assessed using novel methods developed for meta-analysis of genome scans. This collaboration is the largest and most comprehensive analysis of linkage samples involving a psychiatric disorder to date. We demonstrate that combining original genome scan data is a powerful approach for the elucidation of linkage regions underlying complex disease. Our results establish genomewide significant linkage to bipolar disorder on chromosomes 6q and 8q, providing solid information to guide future gene finding efforts relying on fine-mapping and association approaches.

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Multiple QTLs Influencing Triglyceride and HDL Cholesterol Levels Identified in Families With Atherogenic Dyslipidemia. *Y. Yu[1], D.F. Wyszynski[1], D.M. Waterworth[2], S.D. Wilton[3], P.J. Barter[4], Y.A. Kesäniemi[5], R.W. Mahley[6], R. McPherson[7], G. Waeber[8], T.P. Bersot[6], Q. Ma[1], S.S. Sharma[2], D.S. Montgomery[2], L.T. Middleton[2], S.S. Sundseth[2], V. Mooser[2], S.M. Grundy[9], L.A. Farrer[1].* 1) Boston University; 2) GlaxoSmithKline; 3) Australian Neuromuscular Research Institute; 4) The Heart Research Institute, Australia; 5) University of Oulu; 6) Gladstone Institute of Cardiovascular Disease; 7) University of Ottawa Heart Institute; 8) Lausanne University Hospital; 9) University of Texas Southwestern.

High triglyceride (TG) and low high density lipoprotein cholesterol (HDL-C) levels are prevalent features of the metabolic syndrome and increase risk for cardiovascular disease. We conducted a genome-wide scan (448 STR markers) using variance components linkage analysis to localize quantitative-trait loci (QTLs) influencing TG and HDL-C levels in 3071 subjects from 459 families with atherogenic dyslipidemia (TG > 75th percentile and HDL-C < 25th percentile). To obtain proper marker allele frequencies, families were classified on the basis of their ethnic background as European, Finns, and Turkish. The most significant evidence for linkage to TG levels was found in a subset of Turkish families at 11q22 (LOD=3.34) and at 17q12 (LOD=3.44). We carried-out sequential oligogenic linkage analysis to examine if multiple QTLs jointly influence TG levels in the Turkish families. These analyses revealed loci at 20q13 which showed strong epistatic effects with 11q22 (conditional LOD=3.15) and 7q36 which showed strong epistatic effects with 17q12 (conditional LOD=3.21). We also found linkage on the 8p21 region for TG in the entire group of families (LOD=3.08) with most evidence contributed by the European families (LOD=2.80). For HDL-C levels, evidence of linkage was identified on 15q22 in the Turkish families (LOD=3.05) and on 5p15 in the entire group of families (LOD=2.83). These QTLs provide important clues for the further investigation of genes responsible for these complex lipid phenotypes. These data also indicate that a large proportion of the variance of TG levels in the Turkish population is explained by the interaction of multiple genetic loci.

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Identification of new loci for early onset obesity by a genome wide 10K SNP scan in 298 nuclear families from Germany. *F. Ruschendorf[1], K. Saar[1], C. Becker[2], A. Hinney[3], K. Reichwald[3], G. Brönner[3], S. Friedel[3], A. Scherag[4], P. Nürnberg[2], J. Hebebrand[3].* 1) Gene Mapping Center, Max-Delbrueck Center, Berlin-Buch, Germany; 2) Cologne Center for Genomics, University of Cologne, Germany; 3) Department of Child and Adolescent Psychiatry, Rheinische Kliniken Essen, University of Duisburg-Essen, Essen, Germany; 4) Institute of Medical Biometry and Epidemiology, Philipps-University Marburg, Germany.

Background & Aims: Several genome scans have been performed for adult obesity but only one for childhood and adolescent obesity. Our previous scan was performed with a small number of families and a low-density marker set. Therefore, we decided to recruit more families with obese children in order to replicate the study with a high-density marker panel. This should enable us to identify loci particularly involved in early-onset obesity. Methods: A total of 298 families with two or more obese children were collected (1235 individuals). DNA samples were genotyped using the Mapping 10K version 2 from Affymetrix (10204 SNPs). Data were analysed using the programs ALOHOMORA, Genehunter, Genhunter-Modscore, Merlin, and Allegro. Results: The average information content of the SNP array scan was 0.97. Within the subset of families (89) previously analysed by microsatellites the higher information content resulted in higher NPL scores for the linkage signals on chromosomes 8 and 11. Based on the whole data set we revealed other regions of linkage on chromosomes 4, 5, 6, 7, 9, 10, 11, 14, 15, 17, and X. Only a few of them coincide with linkage signals from genome scans in adult obesity. The highest NPL score (>3.0) was obtained for a locus on chromosome 17. The second highest value was obtained for chomosome 4 (NPL=2.95). On chromosome 9 the program Modscore revealed a score of about 3 for a recessive model without any parent-of-origin influence. Conclusion: Our data suggest particular gene loci to predispose to early-onset obesity rather than the adult form while others seem to be involved in both, early- and late-onset obesity.

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Dilated Cardiomyopathy with Ataxia (DCMA) syndrome, a novel autosomal recessive Barth syndrome-like disorder, maps to chromosome 3q. *K. Davey[1], J.S. Parboosingh[1], D.R. McLeod[1], A. Chan[2], R. Casey[1], P. Ferreira[3], F.F. Snyder[1], P.J. Bridge[1], F.P. Bernier[1].* 1) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 3) Alberta Children's Hospital, Calgary, AB, Canada.

Dilated Cardiomyopathy with Ataxia (DCMA) Syndrome is a novel autosomal recessive mitochondrial condition in the Canadian Dariusleut Hutterite population. The main clinical features include a severe early-onset dilated cardiomyopathy, long QT syndrome, non-progressive cerebellar ataxia, testicular dysgenesis, mild development delays and growth failure. In addition, all affected patients have elevated 3-methylgluatoconic and 3-methylglutaric acid levels in both plasma and urine. DCMA shares some similarities with the X-linked Barth syndrome and the other classes of 3-methylglutaconic acidurias, however the clinical features and pattern of inheritance distinguish it from these other conditions. Using a homozygosity mapping approach, a complete genome scan was performed on 5 severely affected patients from consanguineous Hutterite families. We identified an extended disease associated haplotype of 23.9 cM on chromosome 3q26.2-q27.3. Fine mapping using additional markers and patients, as well as unaffected family members, narrowed this region to a minimal candidate region of 2.2 megabases of 3q27.2.

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Genome Wide Scan of Exfoliation syndrome in a large Finnish family. *S. Lemmela[1], E. Forsman[2], A. Eriksson[2], H. Forsius[2], I. Järvelä[1,3].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Population Genetics Unit, Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Laboratory of Molecular Genetics, Helsinki University Hospital, Helsinki, Finland.

In Exfoliation Syndrome (XFS) greyish fibril like material is detected on the anterior surface of the lens and along the pupil margin and same kind material is also found in many extraocular tissues. XFS is a confirmed risk factor for open angle glaucoma. XFS is rare under 50 years of age and as highest approximately 30 % among population over 80 in Scandinavia. The etiopathogenesis of XFS is unknown but there is evidence for a genetic component. The mode of inheritance of XFS has proposed to be autosomal dominant with incomplete penetrance or mitochondrial transmission. We have studied the prevalence and inheritance of XFS in the Åland archipelago, in the south-western coast of Finland, since 1961. In a large pedigree XFS seems to be transmitted as a dominant trait with incomplete penetrance. During the last 40 years a total of 76 patients with XFS have been detected including more than 500 ophthalmologically examined persons. We have performed a genome wide scan with 1000 microsatellite markers for a total of 29 patients and 36 other family members belonging to this pedigree. The analysis was made under assumption of an autosomal dominant mode of inheritance with a low phenocopy rate (0.001, 0.999, 0.999) and a rare disease allele frequency (0.0001). Five markers extending a region of 31 cM at chromosome 18q resulted in positive 2-point lod scores: D18S468 (Zmax= 3.446, =1.00), D18S64 (Zmax=1.695, =0.840), D18S1147 (Zmax=1.682, =0.830), D18S1135 (Zmax=1.385, =1.00) and D18S450 (Zmax=1.491, =1.00). In addition, some positive lod score values were obtained at chromosome 17 (D17S849, Zmax=1.911), chr2 (D2S117, Zmax=1.725), chr 5 (D5S2049,Zmax 1.605) and chr X (DXS7108,Zmax=1.519). None of the loci identified in this study overlap with the previously identified genetic loci for primary open angle glaucoma, suggesting different genetic background of these overlapping eye diseases. We speculate that due to isolation of the study population a predisposing gene has enriched to this island.

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A QTL on chromosome 3p influences the tempo of skeletal maturation in Nepali children. *B. Towne[1], J. Blangero[2], D.L. Duren[1], K.D. Williams[1], T. Dyer[2], M.J. Aivaliotis[2], C.R. Cottom[1], S. Lawrence[1], B. Jha[3], J. Subedi[4], J.L. VandeBerg[2], S. Williams-Blangero[2].* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Tribhuvan University Institute of Medicine, Kathmandu, Nepal; 4) Miami University, Oxford, OH.

Knowledge of genetic influences on skeletal development comes mainly from studies of monogenic disorders. Few studies have examined genetic influences on normal variation in skeletal development, and fewer still have examined the skeletal development of children in non-Western populations. We present here results from an initial whole-genome linkage scan for QTL influencing the tempo of skeletal maturation in children from the Jirel ethnic group in eastern Nepal. The Jirel population numbers ~5,000 individuals, most of who are in one large extended pedigree. More than 1,000 Jirel children are in the Jiri Growth Study, and at their annual exam a hand-wrist x-ray is taken from which skeletal age (SA) is estimated. For these analyses, SA data were selected from a subset of 368 Jirel children (182 boys and 186 girls) aged 6 to 18 years who have each been genotyped for ~400 autosomal markers. Each childs SA was subtracted from their chronological age at the time of their exam to obtain an overall measure of their relative skeletal maturation. A variance components-based linkage analysis method was used to analyze these data and obtain multipoint LOD scores. The heritability of tempo of skeletal maturation from ages 6 to 18 years was highly significant ($h^2 = 0.92$; $p < 7x10^{-4}$). Significant linkage was found of tempo of skeletal maturation to markers on chromosome 3p at 27 cM between markers D3S1304 and D3S1263 (LOD score = 3.32). Plausible positional candidate genes in this region include known and as yet unidentified genes involved in the 3p deletion syndrome. Future work will use an expanded sample of genotyped children in the Jiri Growth Study, and will seek to identify specific genes that influence different aspects of normal skeletal development during childhood. Supported by NIH grants HD40377, AI37091, AI44406, and MH59490.

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Fine mapping of Obesity QTL on 5q14 in a Cohort of Type 2 Diabetes Patients from West Africa. *G. Chen, A. Adeyemo, J. Zhou, Y. Chen, C. Rotimi.* Natl Human Genome Ctr, Howard Univ, Washington, DC.

Previous linkage analysis identified QTL for three obesity phenotypes: BMI (LOD = 2.8), FM (LOD = 3.5) and PBF (LOD = 2.5) on 5q14 (Max location = 98cM, 1 LOD score region 74cM ¨C 117cM) in West Africans with Type 2 diabetes (321 siblings and 36 half siblings with 392 primer pairs with average spacing of 8.9cM throughout the genome). In order to localize the loci more precisely, we added 29 markers throughout the linkage region. We found LOD scores 3.66, 4.06, and 3.16 for BMI, FM, and PFM, respectively, Max location 98cM, and 1 LOD score region 75cM ¨C 108cM. In conclusion, our findings provide evidence that obesity susceptibility genes reside on chromosome 5q14.

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Genome-wide SNP chip homozygosity mapping defines critical region for Native American Myopathy. *D. Stamm[1, 2], C. Powell[2], S. Kahler[3], A. Aylsworth[2], K. Deak[1], S. West[1], D. Craig[4], D. Lince[4], D. Stephan[4], J. Gilbert[1], M. Speer[1], Duke University Medical Center.* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Department of Genetics, UNC-CH, Chapel Hill, NC; 3) Little Rock, AR; 4) Translational Genomics Research Institute, Phoenix, AZ.

Native American Myopathy (NAM) is an autosomal recessive congenital myopathy first reported in the Lumbee people of North Carolina (OMIM 255995). Features include congenital weakness, cleft palate, ptosis, and susceptibility to malignant hyperthermia provoked by anesthesia. We hypothesize the affected individuals have alleles that are shared identity-by-descent (IBD) inherited from common ancestry. We used a SNP genome-wide screen on Affymetrix Gene Chip Mapping 10K Array platform that was performed by Translational Genomics (TGen, Phoenix, AZ). The SNP screen demonstrated shared regions of homozygosity between two affected Lumbee individuals from families Duke Family 2118 and Duke Family 2281 in four regions on chromosome 12. To follow up this screen in order to confirm previously identified regions and narrow the candidate interval, twenty-three microsatellite markers were selected to cover the four regions. Markers were prioritized based on the highest heterozygosity values and spacing within the region. The microsatellite marker data narrowed the region of homozygosity to one region spanning D12S398 to D12S1610 of 11.8 MB (7.6 cM) between the two affecteds. The overall results confirm homozygosity in region 12q13.13 to 12q14.2 in the two affected individual, supporting our hypothesis that the affected individuals share genetic material in an IBD fashion. Biologically plausible candidate genes in the 12q13.13 to 12q14.2 region identified by NCBI and Ensembl include integrin 7, PIP5K2C, KIF5A, MLC1SA, among others. We are currently evaluating candidate genes via sequencing to identify the genetic variant responsible for NAM. Sequencing of exons 1-5 and 7-27 in candidate gene integrin 7 showed no evidence for a mutation; exon 6 is in progress. These findings demonstrate the power and utility of SNP screens to perform homozygosity mapping in rare autosomal recessive diseases using only a few affected individuals.

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A novel Gene for Enlarged Vestibular Aqueduct Syndrome (EVA-Syndrome) ? *R. Birkenhager, A.J. Zimmer, T. Klenzner, A. Aschendorff, R. Laszig, J. Schipper.* Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Freiburg, Killianstrasse 5, D-79106 Freiburg.

Background: Pendred syndrome (MIM 274600), an autosomal-recessive disorder is characterized by sensorineural deafness and goiter. This syndrome is one of the most common forms of syndromic deafness. Hearing loss is prelingual in the majority of the cases, only a subset of patients have a progressive hearing loss later in life. The deafness is associated with temporal bone abnormalities ranging from isolated enlargement of the vestibular aqueduct (EVA) to Mondini dysplasia, a more complex malformation that also includes cochlear hypoplasia. Both, EVA and Mondini dysplasia are easily recognized by computer tomography. PDS is caused by mutations in the SLC26A4 gene. This gene product, a transmembrane protein, is called pendrin. Functional studies in Xenopus laevis oocytes and Sf9 cells have shown that pendrin is a transporter of iodide and chloride. In this study we analyzed several multiplex families with EVA and hearing loss to distinguish between the Pendred- and EVA-Syndrome (MIM 603545). Methods: Individual exon and intron transitions of the SLC26A4 gene of patients were PCR amplified. Direct automatic sequencing of variant fragments was performed with the same primers, on an automatic genetic analyzer. A genomewide linkage analysis was undertaken using the Affymetrix 10K GeneChip mapping 10K Xba SNP array. Results: We identified 15 different mutations in the SLC26A4 gene so far, 6 of these mutations are novel. In 30 % of our patients we could not identify any mutation. These patients carry potential mutations in regulatory domains such as promotor regions, or alternatively the possibility of a distinct locus for a gene of autosomal recessive deafness with enlarged vestibular aqueduct. With a genomewide linkage analysis it was possible to identify genome regions where a gene is located which is, in addition to the SLC26A4 gene, responsible for the development of the Enlarged Vestibular Aqueduct Syndrome. Conclusions: Our results indicate evidences of a second gene which is involved in the development of the Enlarged Vestibular Aqueduct Syndrome.

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High-resolution SNP maps in the genomic regions corresponding to drug-related genes. *A. Iida[1], S. Saito[2], A. Sekine[3], Y. Nakamura[1,2,4].* 1) Laboratory for Pharmacogenetics, SNP Research Center, RIKEN, Tokyo, Japan; 2) Laboratory for SNP Analysis, SNP Research Center, RIKEN, Tokyo, Japan; 3) Laboratory for Genotyping, SNP Research Center, RIKEN, Yokohama, Japan; 4) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

One of major goals in our laboratory is to establish 'gpersonalized medicine'h on the basis of single nucleotide polymorphisms (SNPs) regarding responsiveness or adverse effects to drugs and susceptibility to common diseases. To archive this aim, we have been two different screening programs to detect genetic variations in the Japanese population. One approach was intended to identify up to 150,000 SNPs within transcription units in the Japanese population; more than 195,000 SNPs have been identified already and that information is freely available via our worldwide website (http://snp.ims.u-tokyo.ac.jp/). Second, we have focused on genetic variations in the genomic regions that corresponding to genes encoding drug metabolizing enzymes, transporters, and G-protein coupled receptors. So far, we constructed comprehensive SNP maps of 265 gene loci that contain more than 7,500 genetic variations. Here we introduce the molecular features of each of those loci, such as regional distributions of SNPs, non-synonymous substitutions, insertion/deletion polymorphisms within exonic sequences, and genetic variations of other types. This database constructed in our laboratory should provide a fundamental molecular basis for understanding the pharmacokinetics or pharmacodynamics of drugs used to treat individual patients.

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Pooled DNA sequencing: A fast, reliable and cost-effective method for comprehensive analysis of common SNPs in genetic association studies. *V. Kodavali[1], A. Northup[1], Y. Joo[1], L. Pless[1], J. Wood[1], B. Devlin[1], V.L. Nimgaonkar[1, 2].* 1) Department of Psychiatry; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15213.

Introduction: We have shown that re-sequencing is a precise method for estimating SNP allele frequencies in pooled DNA. We have now adapted this method for genetic association studies. Our strategy involves rapid, comprehensive screening of all common polymorphisms in a defined region by pooled DNA sequencing. Suggestive associations are followed up by individually genotyping SNPs using the samples composing the DNA pools. If confirmed, the associations are tested further in independent samples. Here, we report genetic analysis of ACSL6 (Acyl-CoA synthetase long-chain family member 6, 5q31, 43kb) and SIRT5 (sirtuin 5, 6p23, 45kb), which are involved in the altered phospholipid metabolism and cell signaling and thus may mediate schizophrenia pathogenesis. Methods: DNA pools were made by measuring DNA concentrations using a Pico Green reagent. Pools of cases (n=200) and controls (n=200) were amplified in quadruplicate using the PCR. Peak heights of sequencing traces were used to estimate allele frequencies and were obtained from ABI Prism Sequencing Analysis software version 3.7. Resutls: We identified 38 SNPs at ACSL6 and 31 SNPs at SIRT5 genes, by pooled sequencing (minor allele frequency >10%). Comparison of traces from pooled samples of cases and controls revealed suggestive differences for nine SNPs, following comparisons of variances and estimated error rates. These SNPs were genotyped in individual samples (n=400). We thus detected suggestive trends for association with ACSL6 (rs 11743803, p values allele-wise =0.02 and genotype-wise =0.06). In an extended sample (448 cases and 554 controls) the association remained marginally significant. Conclusions: Our study clearly demonstrates the application of pooled DNA sequencing in identifying and estimating reliable SNP allele frequencies in case-control association studies. We conclude that pooled DNA sequencing is a fast, cost-effective and reliable approach for comprehensive analysis of defined chromosomal regions in association studies.

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Diplotype trend regression (DTR) analysis on ADH gene cluster and ALDH2 gene in alcohol and drug dependent patients. *X. Luo[1,2], H.R. Kranzler[3], L. Zuo[1,2], S. Wang[4], J. Lappalainen[1,2], J. Gelernter[1,2].* 1) Dept Psychiatry, Yale Univ Sch Medicine, New Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Dept Psychiatry, Univ CT Sch Med, Farmington, CT; 4) Dept Biostatistics, Columbia Univ Mailman Sch Pub Health, New York, NY, USA.

The alcohol metabolizing enzymes are genetically and functionally complex. The association of some alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes with alcohol dependence (AD) has been studied in many populations, generally with limited marker sets. To test for associations between six ADH genes, the ALDH2 gene, and AD and drug dependence (DD) in European-Americans (EAs) and African-Americans (AAs), we used a novel regression method, which includes an extension of the structured association (SA) method. Sixteen markers within the ADH gene cluster (including ADH1A, ADH1B, ADH1C, ADH5, ADH6 and ADH7) on chromosome 4, four markers within ALDH2 on chromosome 12, and 38 unlinked ancestry-informative markers (AIMs) were genotyped in a case-control sample of 925 subjects. All markers were in HWE in controls, but 11 markers were in Hardy-Weinberg Disequilibrium (HWD) in some case groups. Diplotype Trend Regression (DTR) analysis (Zaykin et al. Hum Hered. 2002;53:79-91), which allows departures from HWE, controls for population stratification and admixture effects, and takes gene-gene interaction into account, showed that ADH5 and ADH6 genotypes, and diplotypes of ADH1A, ADH1B, ADH1C, ADH7 and ALDH2, were associated with AD or DD in EAs and/or AAs. Each of these genes had at least one marker with a peak [a measure of linkage disequilibrium (LD)] that could localize risk alleles. We found that several genes encoding ADH isoforms, and the ALDH2 gene, were associated with both AD and DD. Some associations between genes and diseases are consistent in direction across different populations and phenotypes, while some are in opposite directions or are population-specific and phenotype-specific. LD studies showed that although there is substantial within-gene LD and limited intergene LD within the chromosome 4 cluster, these results are best explained by 7 independent risk genes.

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Gene-environment interaction between matrix metalloproteinase-3 (MMP3) and education influences the risk for high myopia in Taiwanese. *S. Juo[1,4], C. Liang[2], K. Hung[3], A. Sun[3], H. Wang[2].* 1) Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Clinical Medical Sciences, Chang Gung University, Taiwan; 3) Department of Surgery, Chang Gung Memorial Hospital, Taiwan; 4) Genome Center, Columbia University, New York, NY.

Purpose: We conducted a case-control study to investigate the relationship between the MMP2, MMP3 and TIMP1 genes and high myopia. Methods: We recruited 225 high myopes (-6 D) as cases and 259 controls (-1.5 D). We genotyped 22 SNPs at the three gene, which includes 5 SNPs of the MMP3, 9 of the MMP2 and 8 of the TIMP1 genes. Two commonly studied functional promoter polymorphisms (-1612 5A/6A at MMP3 and -1306 C/T at MMP2) were included in our SNP list. Allele frequencies were tested for the Hardy-Weinberg equilibrium (HWE). The degree of LD was assessed by D and haplotype blocks were reconstructed using the HaploView program, and haplotype analysis was conducted by using PHASE. The association between genotypes and high myopia was assessed for each SNP. We also analyzed the data separately by the college education level as a test of gene x environment interaction. Results: The genotype distribution was not deviated from HWE in any SNP. Eight SNPs were monomorphic, and were excluded for further analyses. The pair-wise D was > 0.9 for each pair of SNPs. The overall genotypic or allelic distribution between cases and controls was not significant for any of the 14 SNPs. In the test of a gene-environment interaction, we found a significant p value of 0.02 for the association between the three genotypes of the 5A/6A polymorphism at MMP3 and high myopia among the subjects with college or higher education. The OR for 6A/6A compared with (5A/6A + 5A/5A) was 2.3(95%CI:1.2-4.2,p=0.008) for high myopia in the subjects of high education. The OR was not significant for subjects without college education. Haplotype analysis did not improve the statistical significance. Other SNPs did not yield significant interactions with education. Conclusion: The present study indicated that a potentially functional promoter polymorphism of the MMP3 gene may confer susceptibility to high myopia under a heavy near-working condition.

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Genetic variation in genes related to endocrine function in European men. *S. John[1], S. Pye[1], M. Jiang[9], A. Pickles[1], G. Bartfai[2], I. Feges[2], F. Casanueva[3], M. Lage[3], G. Forti[4], L. Petrone[4], A. Giwercman[5], K. Kula[7], M. Punab[6], P. Korrowitz[6], S. Boonen[8], H. Borgs[8], R. Walczak-jedrzejowska[7], A. Silman[1], F. Wu[1], I. Huhtaniemi[9].* 1) Univ of Manchester, UK; 2) Univ of Szeged, Hungary; 3) Univ of Santiago de Compostela, Spain; 4) Univ of Florence, Italy; 5) Lund Univ, Sweden; 6) Univ of Tartu, Estonia; 7) Univ of Lodz, Poland; 8) Univ of Leuven, Belgium; 9) Univ of Turku, Finland.

The European Male Aging Study (EMAS) is a prospective random general population cohort study aimed at identifying risk factors which predispose to symptoms and diseases associated with aging. 3,200 men have been recruited in 8 countries. The aim of the current report was to determine the extent of genetic variation across Europe in 12 genes related to endocrine function. 76 SNPs (MAF >5%); in 12 genes (AR, CYP17, CYP19, ESR1, ESR2, IGF1, LHB, LHR, NPY, SHBG, SRD2A5 and VDR) were genotyped in 92 men from each country (n=736). The distribution of genotypes between countries was compared using a 2 test, linear regression was used to test for association between genotypes and height, weight or BMI and GLLAMM equations were employed to account for mean and variance differences between countries. 27 (35%) of SNPs in all genes except NPY and SHBG showed genotype differences between countries ($p < 0.05$). Pair-wise LD analysis appeared broadly similar between populations. However, there were differences in haplotype frequencies for some genes e.g. LHR, the most common haplotype in the Spanish and Italian populations had a frequency of 20%, but a frequency of < 8% in all other countries. 11 of these 27 SNPs were associated with height, weight and or BMI using linear regression with traits adjusted for age. However only 2 associations remained significant (ESR2, SRD2A5) after accounting for country, suggesting these results may be due to the presence of population substructure. These data emphasize the importance of understanding potential population substructure in genetic association studies even in the context of a purely European study group. The findings may partly explain why phenotypic associations of SNPs show variability between different populations.

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A constrained likelihood approach to marker-trait association studies. *K. Wang[1], V.C. Sheffield[2].* 1) Program in Public, Health Genetics, Univ Iowa, Iowa City, IA; 2) Department of Pediatrics, Howard Hughes Medical Institute, Univ Iowa, Iowa City, IA.

Marker-trait association analysis is an important statistical tool for detecting DNA variants responsible for genetic traits. In such analyses, an analysis model on the mean genetic effects of the genotypes is often specified. For instance, the effect of the disease allele on the trait is often specified to be dominant, recessive, additive or multiplicative. Although it is powerful when the analysis model is correctly specified, it has been found that this model-based approach can have low power when the specified model is incorrect. We introduce an approach that does not require the specification of a particular genetic model. This approach is built upon a constrained maximum likelihood where the mean genetic effect of the heterozygous genotype is required not to exceed those of the two homozygous genotypes. The asymptotic distribution of the likelihood ratio statistic is derived in the case of continuous traits and in the case of dichotomous traits. Simulation study suggests that this new approach has power comparable to model-based methods where the analysis models are correctly specified. This approach uses one marker at a time (single-marker analysis). However, given the latest findings that powerful inferential procedures for haplotype analyses can be constructed from single marker analyses, we expect this approach to be useful for haplotype analyses.

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Family-based association analysis in the presence of linkage (APL) with missing parental genotypes: A computer program for single-locus and haplotype tests. *R-H. CHUNG*[1], *E. MARTIN*[2]. 1) Bioinformatics Research Center, North Carolina State University, Raleigh, NC; 2) Department of Medicine, Duke University Medical Center, Durham, NC.

We have developed software for conducting the family-based association analysis method APL (Martin et al. 2003, AJHG), and present simulations to evaluate modifications of the test and robustness to deviation from assumptions. The APL method is based on observing parental allele transmissions to affected siblings. APL can infer missing parental genotypes using genotypes of siblings and properly adjusts for linkage by considering identity-by-descent parameters. In the APL software we used a bootstrap variance estimator, instead of the original robust variance estimator, to offer flexibility in handling different family structures. We further extended the APL algorithm from single-marker to haplotype analysis and provide a global test for haplotype association. We present simulations to verify that the new tests have the correct type I error rate. Power simulations were used to compare the APL to two alternative family-based association methods, PDT and FBAT/HBAT. The APL test consistently showed more power for both single-marker and haplotype analyses using nuclear family data sets. The APL statistic is normally distributed when the number of informative families is large, but this approximation may not be appropriate when the number of informative families is small. Our simulation results showed that rare alleles or rare haplotypes can cause inflated type I error rate for APL tests. We used simulations to provide practical guidelines for when the approximation is valid. In general, using statistics with estimated variance greater than 5 gives reasonable type I error rates. We also explored the effect of violating the assumption of Hardy-Weinberg Equilibrium in allele and haplotype tests. We simulated several data sets with different degrees of departure from HWE. We found that APL is not very sensitive to the departure and remains a valid test. This study shows that the APL software provides a robust and reliable tool for association analysis using single loci or haplotypes in family data.

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Population Association Study of NCAM, ANKK1 and DRD2 Genes with Alcohol Dependence. *B. Yang[1,2], H. Kranzler[3], H. Zhao[4], JR. Gruen[5], X. Luo[1,2], J. Gelernter[1,2].* 1) Dept Psychiatry Yale Univ Sch Medicine, New Haven, CT; 2) VA CT Healthcare System, West Haven, CT; 3) Univ CT Health Center, Farmington, CT; 4) Dept EPH Yale Univ Sch Medicine, New Haven, CT; 5) Dept Pediatrics Yale Univ Sch Medicine, New Haven, CT.

DRD2 is a functional candidate gene for substance use disorders (SUDs), including alcohol dependence (AD), and several DRD2 markers, and an ANKK1 marker previously thought to map to DRD2 (rs1800497, previously known as DRD2 TaqI A) have been associated to various SUDs. DRD2 also maps molecularly close to NCAM1 (which encodes a neural cell adhesion molecule and is also a functional candidate for SUD risk). We therefore conducted a case-control association study of 743 European Americans (325 with AD, 418 controls) and SNPs mapped to the NCAM-ANKK1-DRD2 cluster. . We selected 7, 4, and 4 SNPs in NCAM, ANKK1 and DRD2, respectively, including two DRD2 SNPs known to be functional, and DRD2 TaqI D (rs1800498), DRD2 TaqI B (rs1079597), to investigate their possible association with AD. All SNPs were in Hardy Weinberg equilibrium. Individual SNPs associated with AD at the $p<0.05$ significance level were selected for haplotype analysis. The associated SNPs were ANKK1 (rs4938016, $p = 0.018$), DRD2 TaqI D (rs1800498, $p = 0.045$), and DRD2^141ins/del (rs1799732, a functional promoter variant, $p = 0.0089$). Although DRD2^C957T (rs6277) showed borderline significance ($p = 0.06$), it was also included in the haplotype analysis since it affects mRNA stability. After adjusting for sex ($p = 3.88 \times 10\text{-}12$) and age (p close to 0), the generalized linear model analysis of the four-SNP haplotype, consisting of ANKK1 (rs4938016)-DRD2^C957T-DRD2 TaqI D-DRD2^141ins/del, resulted in two significantly associated haplotypes, 1-1-1-1 (frequency=0.021, OR=5.53, CI=[4.29, 6.77], p=0.0069) and 2-2-2-2 (frequency=0.182, OR=1.56, CI=[1.16, 1.95], p=0.027). Population stratification was tested using a panel of 36 Short Tandem Repeat (STR) markers that we designed to differentiate major US populations, and was ruled out as a source of spurious association. We conclude that ANKK1 and functional DRD2 variants are associated to AD.

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Candidate-gene association study of mothers with Pre-Eclampsia and their offspring, analyzing 758 SNPs in 187 genes. *K.A.B. Goddard[1], G. Tromp[2], R. Romero[4], J.M. Olson[1], Q. Lu[1], Z. Xu[1], J.K. Nien[4], R. Gomez[4,5], E. Behnke[5], M. Solari[5], J. Espinoza[4], C.J. Kim[4], J. Santolaya[4], T. Chaiworaspongsa[4], Y.M. Kim[4], G. Lenk[2], K. Volkenant[2], H. Kuivaniemi[2,3].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Center for Molecular Medicine & Genetics; 3) Dept of Surgery, Wayne State Univ, Detroit, MI; 4) The Perinatology Research Branch, NICHD, NIH; 5) The Center for Perinatal Diagnosis & Research, Sotero del Rio Hospital, P. Universidad Catolica de Chile, Santiago, Chile.

Pre-eclampsia (PE) affects 5-7% of pregnancies worldwide and is a leading cause of maternal death and perinatal morbidity and mortality. Genetic factors may account for more than half of the variability in liability to PE. We conducted a large-scale association study with candidate genes selected for a potential role in obstetrical complications. SNP discovery was performed by DNA sequencing at Genaissance Pharmaceuticals, Inc (New Haven, CT). Genotyping was carried out at Genaissance Pharmaceuticals, Inc. using the MassARRAY System (Sequenom, Inc., San Diego, CA). Women with PE (n=426) and their offspring (n=323) were compared with control women (n=512) and their offspring (n=486) from the same hospital-based population. Haplotypes were constructed using the EM algorithm, and empirical p-values were obtained for a logistic regression-based score test, adjusted for maternal age and offspring gender. Interaction and incompatibility models between maternal and offspring genotypes were evaluated. The maternal *LPL* (p=.0077), *TNF* (p=.009), and *COL1A1* (p=.041) genotypes and the offspring *REN* (p=.0067) genotype were associated with PE. Common candidate genes for PE, including *MTHFR* and *NOS3*, were not significantly associated with PE. For the interaction model, *PROS1* (p=.0008) and *FGF4* (p=.0009) gave the most significant results. For the incompatibility model, *VWF* (p=.0004) was associated with PE. This is one of the most comprehensive genetic association studies of PE to date. Identification of the genetic regulators of PE may have broad implications, since women with PE are at increased risk of cardiovascular diseases later in life.

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Comparison of Linkage Disequilibrium Measurement Programs. *M. Britain, D.L. Koller.* Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN.

Linkage disequilibrium (LD) between markers partly determines the placement of SNPs in the testing of candidate genes. Several programs are available to determine the extent of pairwise LD. Because the same data can produce variable results depending on the program used, comparison of LD programs was necessary.

Using Simla (v. 3.0), we simulated pedigree files consisting of 800 three-generational families. We specified biallelic markers, equally spaced. We compared the accuracy and precision of two programs for computing D as a measure of LD, Haploview (v. 3.11) and the package consisting of SimWalk2, HaploXT, and GOLD. SimWalk2 (v.2.82) was used to generate haplotypes, which were then used by Haploxt, (part of the GOLD software package, v. 1.1, Abecasis et al) to compute D.

In the extreme case of no LD (D=0), Haploview and Simwalk2/HaploXT generated mean D values of 0.02 and 0.026, respectively. With complete LD (equal to 1), Haploview and Simwalk2/HaploXT produced values of 0.99 and 1.0, respectively.

We then simulated an intermediate value of D. The four haplotype frequencies were 0.60, 0.30, 0.05, and 0.05, with SNP minor allele frequencies each of 0.35, leading to a D of 0.78022. For n = 10, the mean D values for Haploview and Simwalk2/HaploXT were 0.78 (SD = 0.009) and 0.804 (SD = 0.021), respectively. With respect to the true value of LD, Haploview was more accurate than Simwalk2/HaploXT. The mean D from Haploview was not significantly different from the simulated value ($p = 0.9412$). The mean D from Simwalk2/HaploXT was significantly different from the simulated value ($p = 0.0005$). In addition, Haploview was more precise, with a more narrow dispersion of values. Values calculated in Haploview ranged from 0.77 to 0.79 (variance = 8.89 x 10-5) while values calculated in Simwalk2/HaploXT ranged from 0.778 to 0.837 (variance = 4.64 x 10-4).

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Linkage Disequilibrium Patterns in Inbred Mice and Implications for Fine Mapping of Disease Loci. *D. Wang, M. You.* Department of Surgery and the Siteman Cancer Center, Washington University, St Louis, MO.

Linkage disequilibrium (LD) patterns have been extensively studied as guidance for fine mapping of disease loci in humans. However, less attention has been paid to LD patterns in the population of inbred mice. Inbred mouse population has a very unique history owing to the fact that each inbred mouse strain was a product of artificial selection over 20 generations of inbreeding. In this study, we assessed the LD patterns that exist in the inbred mouse population using the Roche SNP data that contains ~153,140 SNPs for 18 inbred mouse strains. Our results show that average pairwise LD (r2, squared correlation coefficient of alleles at two loci) decays significantly over the first 32 kb of the distance range, which seems to be larger than observed in humans. Large variability was also observed for pairwise LD vs. physical distance. SNPs with similar allele frequencies have stronger LD than those with different allele frequencies, and SNPs with higher allele frequencies also tended to have stronger LD. With the cladistic method implemented in software CLADH, we also show that LD mapping for a dichotomous phenotype with high (~80%) to complete penetrance has sufficient statistical power and accuracy. However, LD mapping has only limited use in mapping a mutation with moderate penetrance (~60%) when the sample size is small. This study provides the fundamental understanding of LD patterns in the inbred mouse population, and the feasibility and effectiveness of LD mapping of candidate genes using inbred mouse strains.

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The impact of missing and erroneous genotypes on tagging SNP selection and power of haplotype tests. *W. Liu, W. Zhao, G.A. Chase.* Division of Biostatistics, Penn State College of Medicine, Hershey, PA.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic polymorphism in the human genome and serve as effective markers for localizing disease susceptibility genes. With the recent development of sequencing technology, the number of SNP markers is increasing so quickly that current genotyping technologies are inadequate for genotyping all available SNP markers for a typical linkage/association study. Much attention has recently been paid to methods for selecting the minimal informative subset of SNPs in identifying haplotypes, but there has been little investigation of the effect of missing or erroneous genotypes on the performance of these SNP selection algorithms and haplotype tests using selected tagging SNPs. Even with modern automated DNA analysis methods, the problem of missing allelic data or genotyping error is not uncommon. In this project, we explored the effect of missing genotype or genotyping error on the performance of three tagging SNP selection programs: Claytons diversity based program **htstep**, Carlsons linkage disequilibrium (LD) based program **ldSelect**, and Strams coefficient of determination based program **tagsnp.exe**. When randomly selected known loci were relabeled as missing, we found that the average number of tagging SNPs selected by all three algorithms changed very little and the power of subsequent haplotype tests using the selected tagging SNPs remained close to the power of these tests in the absence of missing genotype. When randomly selected known loci were converted to another genotype, we found that the average number of tagging SNPs selected by all three algorithms increased. Strams program had the largest and Claytons program had the smallest increase. The power of the haplotype tests from all three programs decreased quickly with the presence of genotyping error. These results have direct implications for designing genome-wide association studies.

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Association study of Estrogen receptor-alpha gene with mineral bone density in a group of unrelated Italian females. *G. Malerba[1], E. Petrelli[1], A. Sangalli[1], L. Xumerle[1], V. Braga[2], P.F. Pignatti[1], M. Mottes[1].* 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) Rheumatology Unit, Univ of Verona,, Valeggio S/M, Italy.

Estrogen receptor-alpha (ESR1) gene has been suggested to be a candidate for variability in bone mineral density. We report the study of 2 polymorphisms (PvuII and XbaI) of the ESR1 gene in 657 peri and postmenopausal Italian women. ESR1 polymorphisms were analyzed separately or in haplotype combination. Association analysis of the single markers with BMD values at spine and femur did not show any significant association. Haplotypes were reconstructed and no significant association with BMD values at the 2 distinct sites was observed. Association analysis preformed in a subgroup of individuals having an elevated Body Mass Index (BMI > 35) showed a significant association of PvuII (p=0.04) or XbaI (p=0.005) polymorphism with femur BMD values. Haplotype analysis showed that the PvuII-XbaI T-A haplotype was associated to lower femur BMD values (p=0.01). In conclusion, these data suggest the ESR1 may be involved in BMD variability in women with an elevated BMI. These results should be confirmed in a larger sample.

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Multiplexed Haplotype-Specific Extraction (HSE) of Genomic DNA. *J. Dapprich[1], V. McCarro[2], C. Turino[2], G.S. Scavello[2], N. Murphy[2], D. Monos[3, 4].* 1) Generation Biotech, Lawrenceville, NJ. USA; 2) GenoVision, West Chester, PA. USA; 3) Department of Pathology and Laboratory Medicine, The Childrens Hospital of Philadelphia, Philadelphia, PA. USA; 4) Deptartment of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Haplotype-Specific Extraction, HSE, physically separates a diploid sample into its haploid components using magnetic bead technology without knowledge of familial information. Haplo-separated DNA is then analyzed with kits and assays currently used for DNA-typing. HSE improves the accuracy of tissue typing: It enables unambiguous HLA typing of diploid allele combinations that may otherwise fail to be resolved. Haplotyping can improve the power of Association Studies: The conversion of individual diploid samples into more informative haploid components can increase the power to find correlations between traits and shared variants in case-control studies. Probes for the HLA loci A, B and DRB1 were used to separate DNA fragments from a single tube containing 400 ng genomic DNA of known HLA type on the GenoM-6/EZ-1 HSE robotic system. The haploseparated DNA was amplified in three independent PCRs and confirmed as haploid by HLA-typing with the InnoLiPA reverse SSOP (sequence specific oligonucleotide probe) system. Multiplexed haploseparations provide DNA fragments that can allow the derivation of extended molecular haplotypes. Based on publicly available SNP information it should be possible to separate the entire MHC for one person in one run. The approach is to 1)SNP-genotype candidate regions, 2)Haploseparate based on this information, and 3)Assemble contiguous molecular haplotypes for DNA samples of interest. Routine haplo-separation of large genomic regions can simplify the fine mapping of chromosomal regions for susceptibility genes and validate statistically derived haplotypes. HSE can increase the resolution and quality of genetic analysis in other diagnostic applications such as oncology testing, metabolic profiling and should improve our understanding of multi-factorial diseases and disease classification.

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ALBERT: A Likelihood-Based Estimation of Risk in Trios. *A.A. Mitchell[1], E.S. Emison[2], A. Chakravarti[2], E.A. Thompson[1].* 1) University of Washington, Departments of Genome Sciences and Statistics, Seattle, WA; 2) Johns Hopkins University School of Medicine, Baltimore, MD.

The original transmission disequilibrium test (TDT) developed by Spielman et al, is a popular test for linkage in the presence of association in case-parent trios. Two major problems with the TDT are that its false positive rate is inflated in the presence of undetected genotyping error and it suffers from low power, particularly when the penetrance of the disease of interest is not multiplicative with the number of copies of the risk allele.

Here, we present ALBERT, A Likelihood-Based Estimation of Risk in Trios. ALBERT tests for association between a diallelic marker and a dichotomous trait in case-parent trios. The method, which provides estimates of genotype relative risks, genotyping error rate and risk allele frequency, is more powerful than the TDT for most sample sizes, genotype relative risks, allele frequencies and genotyping error rates. It has a low false positive rate and is robust to the presence genotyping errors. We have implemented the method in a software package, ALBERT, which is freely available through our website.

We applied our method to 29 diallelic markers spanning the RET region on chromosome 10q11.12 in 130 trios ascertained through a child affected with Hirschprung disease. We found a stretch of nine consecutive markers showing significant association ($p < 10^{-4}$) with Hirschprung disease; the middle four of these markers were significant at $p < 10^{-9}$. Thus, ALBERT has shown itself to be an effective tool in identifying alleles associated with increased risk of disease.

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DNA pooling enables cost-effective genome-wide SNP association studies. *A. Gutin, V. Abkevich, K.M. Timms, T. Tran, D. Shattuck, J.S. Lanchbury, M.H. Skolnick.* Myriad Genetics Inc, Salt Lake City, UT.

Recently, remarkable technical advances have occurred that now make it feasible to screen the entire human genome for 500,000 or more SNPs using various microarray-based technologies. A major barrier to widespread application of these technologies is cost. We have developed a robust cost-effective DNA pooling approach to genome-wide SNP analysis. The power to detect an association strongly depends on how precisely the hybridization intensities and the corresponding allele frequencies can be measured. For the Affymetrix Centurion microarray, the standard method of estimating the allele frequencies has a standard deviation (SD) of about 7.4% for a typical SNP. We have developed a new approach to estimate allele frequency with much higher precision. The main idea of this approach is to combine hybridization intensities from different oligo-nucleotide probes corresponding to the same SNP with weights reflecting the quality of the probes. In order to estimate the qualities of different probes, 100 individual DNA samples were run on Affymetrix Centurion microarrays. Within this approach the allele frequency for a typical SNP can be estimated with SD of 2.5%. We have compared the power to detect association using our DNA pooling approach with the power to detect association based on individual genotyping. The following refers to a dataset of 250 cases and 250 controls and a significance level of p-value=0.001. For a SNP with a minor allele frequency of 25%, the minimal detectable allele frequency difference between cases and controls is about 11% using our DNA pooling approach (corresponding to power to detect association of 50%). For the same dataset with individual genotyping, the minimal detectable allele frequency difference is about 9%. This comparison demonstrates that our DNA pooling approach does not significantly reduce the power to detect association. The minimal loss of power using the DNA pooling approach facilitates the widespread use of high content SNP microarrays for a variety of disease association and population genetics applications.

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Identification of translation initiation factor eIF-2B gamma subunit (EIF2B3) on chromosome 1p for age at onset of Parkinson disease: A potential Park10 gene. *J.M. Vance, S.A. Oliveira, X. Qin, X. Huo, M.A. Pericak-Vance, Y.J. Li.* Duke Univ Medical Ctr, Durham, NC.

We previously reported a linkage region on chromosome 1p (LOD=3.41) for genes controlling age at onset (AAO) of Parkinson disease (PD). This AAO linkage peak is essentially identical to a risk linkage peak for PD (PARK10) reported by Hicks et al. (2002) in their study of the Icelandic population, thus providing strong evidence of a major PD gene lying in this region. We recently reported a fine-mapping study, called iterative association mapping, by genotyping 284 SNPs in a 19.2Mb region centered under the AAO linkage peak in a set of 267 multiplex families (at least two affected individuals). Two candidate genes were found to associate with AAO, in which EIF2B3 showed the strongest significant association with AAO (P=0.0003 at SNP rs546354) based on 11 SNPs genotyped in the gene. These results were further supported by families linked to D1S2134, the chromosome 1p linkage peak marker. Here, we followed up this gene in an increased number of multiplex families (308 families). In total, we examined 45 SNPs in the EIF2B3 region including genes located upstream and downstream of EIF2B3, that is, from KIF2C to TESK2. The average distance between markers was about 5kb within a gene. We used the Monks-Kaplan method (Monks and Kaplan 2000) implemented in the QTDT program to test association between markers and AAO. The HBAT program was used for haplotype association testing. We found two clusters of markers in strong LD ($r2 < 0.6$) with significant results. The first large LD-bin with the most significant results ($P< 0.001$) was found between exons 4 and 6 of EIF2B3 and extended to KIF2C. Interestingly, a significant marker (P=0.017) in the second LD-bin was located in the middle of the first LD-bin, between exons 5 and 6. Eight tagSNPs were selected based on a lower r2 threshold ($r2< 0.4$), of which one tagSNP was selected from one LD-bin. The pairwise haplotype analysis shows promising results for tagSNPs selected from these two LD-bins (P=0.002), indicating a potential disease locus that may be located between exons 5 and 6. Further sequencing of these two exons is underway.

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Candidate gene analysis of myopia. *M.E. Cooper[1], S.E. O'Brien[2], K.S. Zandick[3], J.C. Murray[2], M.L. Marazita[1], D.O. Mutti[3].* 1) School Dental Med, Center for Craniofacial and dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) University of Iowa, Department of Pediatrics, Iowa City, IA; 3) Ohio State University, College of Optometry, Columbus, OH.

Myopia is a common condition acquired in childhood. Treatment includes corrective lenses or surgery but in rare cases, myopia can lead to debilitating conditions including retinal detachment and blindness. In this study we examine the role of 7 candidate genes and loci using association and linkage approaches on 123 pedigrees ascertained through one or more myopic individuals. 477 family members were genotyped; 42% of the families have multiple affected siblings (mean typed affecteds per family = 2.2+1.0). Seventeen SNPs in 5 genes and 12 microsatellite markers on chromosomes 4,11,12,18 and 21 were analyzed. Phenotypes analyzed included myopia, myopia with age of onset after 14, and axial length. The results for myopia are reported here; the results with the other phenotypes were similar. Statistical analyses included tests of association (TDT, haplotype TDT, and reverse TDT with tests of Hardy-Weinberg equilibrium to guard against false positives) and tests of linkage-parametric (95% penetrant dominant model with 0.134 disease frequency) and nonparametric. Two of the three SNPs within FGF2 on 4q27 showed significant findings from different analysis methods: one SNP had the common allele over transmitted (p=0.01) with a significant maternal effect (p=0.03), another had a parametric LOD score near 1.0. One SNP within BDNF on 11p14.1 had a parametric LOD of 1.2. On chromosome12, anonymous microsatellite marker D12S2076 (12q21.31) had one allele borderline significantly over transmitted (p=0.08) and another significantly under transmitted (p=0.03); D12S1051 (12q23.1) had an allele significantly over transmitted (p=0.04) and another borderline significantly under transmitted (p=0.06). The results on chromosome 12q are consistent with other myopia studies; our study suggests FGF2 involvement with the development of myopia. Further study to isolate specific etiologic variants is warranted. Support: NIH/NEI grants #U10-EY08893 and R24-EY744701, Ohio Lions Eye Research and EF Wildermuth Foundations.

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Genetic Linkage Study of High-Grade Myopia in an Hutterite Population from South Dakota. *P.C. Paluru[1], S. Nallasamy[2], M. Devoto[3], N.F. Wasserman[1], J. Zhou[1], T.L. Young[1,2].* 1) Division of Ophthalmology and Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Ophthalmology, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Department of Biomedical Research, Nemours Childrens Clinic, Wilmington, DE.

Myopia is a common complex disorder, and severe forms have implications for blindness due to increased risk of premature cataracts, glaucoma, retinal detachment, and macular degeneration. Autosomal dominant (AD) non-syndromic high myopia (myopia 5.00 diopters) has been mapped to chromosomes 18p11.31, 12q21-23, 17q21-23, 7q36, and 2q37.1. Here, we demonstrate linkage for non-syndromic high myopia in a large, Hutterite family to a new locus on chromosome 10q21.1. After clinical evaluation, genomic DNA was genotyped from 29 members of a Hutterite family from South Dakota (7 affected, 9 unknown). The average refractive error of affected individuals was -7.04 diopters (range = -3.75 to -12.25). Prior to proceeding with a genome screen, linkage was excluded for the known AD non-syndromic and syndromic high myopia loci, including Stickler syndrome types 1, 2, and 3; Marfan syndrome; Ehlers-Danlos syndrome type 4; and Juvenile glaucoma. A genome screen was then performed using 382 markers with an average inter-marker distance of 10 cM. SimWalk2 software was used for multipoint linkage based on AD and AR models with a 90% penetrance and a disease allele frequency of 0.001. Additional fine-point mapping yielded a maximum LOD score of 3.22 under an AD model at microsatellite marker D10S1643. Fine-point mapping and haplotype analysis defined a critical region of 2.67 cM on chromosome 10q21.1. Haplotype analysis demonstrated two distinct haplotypes segregating with high myopia, possibility indicative of two distinct mutations occurring in the same gene. A search for genes physically mapped in this region revealed only two known genes: PCDH15 (protocadherin 15) and ZWINT (ZW10 interactor). Mutational characterization of these genes resulted in 23 polymorphisms and none of these segregated with the affection status. We have identified a novel myopia locus, further demonstrating the genetic heterogeneity of this disorder.

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High-density SNP association study of the autism susceptibility loci on chromosome 2q and 7q. *J. Lamb[1], E. Maestrini[2], L. Winchester[1], A. Morris[1], H. Butler[1], E. Bacchelli[2], F. Blasi[2], G. Barnby[1], N. Sykes[1], A.J. Bailey[3], A.P. Monaco[1], IMGSAC.* 1) Wellcome Trust Centre for Human Genetics, Oxford, UK; 2) Dept of Biology, University of Bologna, Italy; 3) University Department of Psychiatry, Park Hospital for Children, Oxford, UK.

Autism is a severe neurodevelopmental disorder, likely to arise on the basis of a complex genetic predisposition. The IMGSAC genome screen for linkage in affected sib-pair families identified two principal loci on chromosomes 2q and 7q, which have been independently replicated. These broad ~40Mb linkage peaks contain ~200 genes. Fine mapping has not significantly narrowed the linked region, and mutation screening of promising candidate genes has failed to conclusively identify any etiological variants. Therefore, we have undertaken a gene-based, high-density SNP typing and association study in the two regions of linkage to identify susceptibility variants.

We used data from the HapMap project to evaluate the distribution of linkage disequilibrium (LD) and haplotype blocks across the two ~40Mb regions. We selected haplotype-tagging SNPs using Haploview and the Gabriel algorithm for block definition from blocks overlapping all known genes and their putative regulatory elements, thereby reducing the amount of genotyping required and capturing the majority of common variation in approximately 80% of all gene regions. Following power calculations to maximize efficiency, we have genotyped 1536 SNPs (including controls for mis-typing and stratification) at each locus using the GoldenGate assay, on a sample of 576 individuals including 192 sex-matched controls and 125 trios from multiplex families selected based on their allele sharing status.

Preliminary bioinformatic analysis has been carried out with regard to SNP distribution, LD block and gap statistics, and intragenic haplotype tagging efficiency. Sample and SNP genotyping success rates are ~99% and 96% respectively. Analysis will be performed on haplotypes within each block using both family-based and case-control association tests, and the results presented.

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Interpreting results from association studies using analytical and permutation based methods. *L. Li[1], P.L. St Jean[1], M.G. Ehm[1], M.E. Weale[2], D.B. Goldstein[3].* 1) Discovery&Pipeline Genetics, GlaxoSmithKline R&D, RTP, NC; 2) Institute for Human Genetics and Health, University College London, Darwin Bdg (Biology), Gower St, London WC1E 6BT, UK; 3) Center for Population Genomics and Pharmacogenetics, Duke Institute for Genome Sciences and Policy, Duke University, DUMC Box 3471, 4006 GSRB II, 103 Research Drive, Durham, NC 27710.

Attrition in drug development is a significant problem leading to escalating costs. Most drug targets are identified via animal models and lack human validation information. The HiTDIP program (high throughput human-disease specific target program) is designed to provide human target validation information for complex diseases in the form of association of SNPs with disease. We genotyped 4267 SNPs in 1405 tractable genes, in 3227 individuals in a two stage case/control design. Genotypic, allelic and haplotypic tests were performed and permutation p-values calculated. We identified genes as associated with type 2 diabetes if at least one test was significant ($p<0.05$) in the primary screen and gene based test was significant in the secondary study. We estimated the experiment false positive rate to be 0.14. We estimated the number of true signals using these data to help us interpret the importance of the results using both analytical and permutation based methods. Results indicate that the chance of identifying true signals is high based on the study design and at least 5 of the significant genes are truly associated with the disease at 5% level.

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Linkage of TB and TNF- to IL10 and TNFR1 gene regions. *C.M. Stein[1,2], L. Nshuti[2,3], R.D. Mugerwa[2,3], D. Leontiev[1], C. Millard[1], W.H. Boom[2], C.C. Whalen[1,2], S.K. Iyengar[1].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Tuberculosis Research Unit, CWRU, Cleveland, OH; 3) Makerere University School of Medicine, Kampala, Uganda.

Tuberculosis (TB) is a growing global public health problem, particularly in the face of the HIV epidemic. Evidence for a role of host genetics in TB susceptibility is accumulating, but association studies of candidate genes have offered inconsistent results. Much of this variability in study results is likely due to inconsistency in phenotype definition and study design. To model the multifactorial nature of TB, we have developed expression of tumor necrosis factor- (TNFa) as an intermediate phenotype, and focused on candidate genes that might be involved in TNFa regulation and TB risk. We analyzed 398 individuals from 66 families from a household contact study in Kampala, Uganda. Culture confirmed disease defined TB disease, and TNFa expression in response to *M. tuberculosis* (Mtb) culture filtrate was measured as a continuous trait. Microsatellite markers were genotyped in 11 candidate gene regions, and model-free multipoint linkage analysis utilized Haseman-Elston (SIBPAL) regression and the conditional logistic affected sibpair (LODPAL) model. We found that the interleukin (IL)-10 gene and TNF receptor 1 (TNFR1) gene regions were linked to both Mtb antigen-induced TNFa expression and TB affection in HIV-seronegative relative pairs at the =.05 level. TNFa demonstrated linkage in the SIBPAL analysis (IL10: D1S256 p=.03, TNFR1: D12S1625 p=.05). Similar results were found using the LODPAL models (D1S256 LOD=1.35, D12S1625 LOD=.74). In addition, the interferon-gamma receptor 1 gene (IFNGR1) was linked to TB status using both statistical models (D6S1587 LOD=2.35 from LODPAL, D6S1675 p=.03 from SIBPAL). IL-10 and IFNGR1 have been associated with TB in previous molecular studies, but this relationship between TNFR1 and TB has never been demonstrated in a human population. Other candidate genes showed linkage to TB, but only in the conditional logistic analysis. Further analyses will be conducted to elucidate the susceptibility alleles and the direction of effect on TNFa expression.

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Re-analysis of the AGRE autosomal linkage scan to account for potential imprinting effects. *M.D. Fallin[1], K. Cheslack-Postava[1], A. Feinberg[2], C. Newschaffer[1].* 1) Dept Epidemiology, Johns Hopkins Univ, Baltimore, MD; 2) School of Medicine, Johns Hopkins University, Baltimore, MD.

Although evidence clearly suggests that genetic factors play an important role in autism, no autism-related genes have been conclusively identified. This is likely due to a complex etiology that involves genetic, epigenetic, and environmental influences during development. Epigenetic influences, such as imprinting, have been implicated in related disorders including Angelmans syndrome and fragile X, although evidence for imprinting effects in autism is still equivocal, with findings of both paternal or maternal inheritance, depending on the study and genomic region. If imprinting is an important mechanism, linkage analyses that incorporate parent-of-origin effects should be a fruitful way of identifying regions that harbor autism genes. With this in mind, we have applied both parametric and allele-sharing linkage analyses that model imprinting to the genome scan data generated for the Autism Genetics Resource Exchange (AGRE) samples (1). We present results for GENEHUNTER-IMPRINTING (2) and GENEFINDER-POO (3) for all 22 autosomes in 195 families with at least two affected siblings where cases are defined as autism disorder, and 303 families with >1 case defined as autism, not quite autism, or a broad autism phenotype. Our strongest initial finding is on chromosome 5p under a maternal expression model, with an HLOD = 4.68 and a 1 LOD interval of 14.4 cM, according to parametric analyses. Additional findings are on chromosomes 7, 19, and 20. References: 1. Yonan et al (2003) Am J Hum Genet 73:886. 2. Strauch et al (2000) Am J Hum Genet 66(6):1945-57. 3. Liang KY et al (2001)Hum Hered 51: 64-78.

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Linking candidate-genes to orofacial clefts in Lithuanian population. *V. Kucinskas[1], A. Morkuniene[1], D. Kasperaviciute[1], A. Utkus[1], L. Linkeviciene[2].* 1) Dept Human & Medical Genetics, Vilnius Univ, Vilnius, Lithuania; 2) Institute of Odontology, Vilnius Univ, Vilnius, Lithuania.

Nonsyndromic cleft lip and/or palate is one of the most common craniofacial malformations. It is composed of two separate entities: cleft lip with or without cleft palate (CL+/-P) and cleft palate only (CP). Both have a genetic background, and environmental factors probably disclose these malformations. We used the transmission/disequilibrium test (TDT) to investigate the relationship between orofacial clefts and microsatellite and biallelic markers associated with five candidate genes: *TGFA*, *TGFB3*, *RARA*, *GABRB3* and *BCL3* in the patients from Lithuania. 86 case - parent trios were ascertained through a child with a nonsyndromic oral cleft: 62 trios with the patient with cleft lip and palate (CLP), 10 trios with cleft lip (CL) and 14 trios with cleft palate (CP). Association was found between microsatellite D2S292 allele 6 (182 bp) in the *TGFA* gene and CL+/-P (TDT for allele - wise analysis: ^{2}= 16,808, 8 df, p = 0,032; TDT for genotype - wise analysis: ^{2}= 31,178, 19 df, p = 0,039). No association was found for this marker when all patients (CL+/-P and CP) were analyzed together, suggesting different role of *TGFA* gene in CL+/-P and CP. The inheritance of *Taq*I marker alleles (C1 allele is reference and C2 - variant, i.e. deletion of 4 bp in intron V) of the *TGFA* locus was analyzed in the sample of 40 informative case-parent triads out of 86 initially genotyped ones. We evaluated the presence of association between *Taq*I marker alleles in the *TGFA* gene and the risk of isolated CL+/-P. Our data provide no evidence of the role *TGFA* genotype in the *Taq*I site and susceptibility to cleft lip and/or palate. There is no evidence of significant linkage disequilibrium with the C2 allele in *Taq*I site and CL+/-P phenotype. No significant differences were found when comparing data by clefting type.

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MHC association in multiple sclerosis is restricted to the HLA class II region by dense SNP mapping. *M.R. Lincoln[1], A. Montpetit[2], M.Z. Cader[1], J. Saarela[3], D.A. Dyment[1], M. Tiislar[2], V. Ferretti[2], P.J. Tienari[4], N.J. Risch[5], A.D. Sadovnick[6], L. Peltonen[3], G.C. Ebers[1], T.J. Hudson[2].* 1) Dept. of Clinical Neurology, University of Oxford, Oxford, UK; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 3) Dept. of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 4) Dept. of Neurology, University of Helsinki, Helsinki, Finland; 5) Center for Human Genetics, University of California San Francisco, San Francisco, USA; 6) Dept. of Medical Genetics and Faculty of Medicine (Division of Neurology), University of British Columbia, Vancouver, Canada.

Multiple sclerosis is the most common neurological disease affecting young adults in Northern European populations. The strongest candidate for genetic susceptibility is the MHC, particularly HLA-DRB1, where linkage and association have been consistently reported. There is evidence, however, for locus and allelic heterogeneity within the MHC and an important question has been whether HLA-DRB1 itself, nearby genes in the extended MHC, or combinations of these loci, underlie MS susceptibility. We attempted to resolve this challenging question by genotyping 4,203 individuals from two large Canadian and Finnish cohorts totaling 1,185 MS families with a high-density SNP panel spanning the MHC and flanking regions. Haplotype blocks were identified and assessed for association with MS. The strongest associations in Canadian samples were with blocks within the HLA class II region ($P4.9\times10^{-13}$), but none were greater than that of HLA-DRB1 itself ($P2.7\times10^{-13}$). These findings were corroborated in the Finnish cohort ($P1.8\times10^{-16}$). Conditioning on either HLA-DRB1 or the most significant HLA class II haplotype block, we observed no additional block or SNP association to suggest an MHC contribution to susceptibility independent of HLA-DRB1. This study therefore strongly argues that MHC-associated susceptibility to MS is determined by HLA-DRB1 alleles, their interactions and/or closely neighboring variants.

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Whole genome association study for psoriasis using the Quebec LD Map custom genotyping chip. *J. Raelson, P. Croteau, Q. Nguyen Huu, B. Paquin, P. Van Eerdewegh, R. Little, J. Segal, B. Stojkovic, H. Fournier, R. Allard, V. Perepetchai, T. Nguyen, N. Laplante, J-M. Vidal, J. Hooper, T. Keith, A. Belouchi.* Genizon Biosciences, Vilee-St-Laurent (Montreal), PQ, Canada.

We previously performed a whole genome association study (WGAS) for Crohns disease using 500 individually genotyped trios from the Quebec Founder Population (QFP) and 165,000 SNP markers distributed evenly across the genome at a density of approximately 1 marker per 17 kb. We were able to detect highly significant association at the CARD15 locus, which has a lower extent of LD than 70 percent of the genome, using a SNP density as low as 1 marker per 47 kb for our QFP sample. We then mapped variation in LD across the genome using a multi-marker metric of LD and control haplotypes from the Crohn's study and used this information to construct the ~ 81,000 Quebec LD marker map (QLDM) with marker spacing of 1 SNP per 30 kb at LD levels equal to or greater than that of CARD15 and with increasing density as high 1 SNP per 10 kb in regions that were identified as having a lower extent of LD.

The QLDM was then used to perform a WGAS for psoriasis using 500 trios with affected members from the QFP. Fifteen hundred individuals were genotyped using a custom chip created by Perlegen Sciences for Genizon Biosciences containing the QLDM markers. We identified 17 regions that were associated with psoriasis at significance of P less than 10^{-5}, including the previously reported PSORS1 locus in the HLA region of 6p21, which was associated at a significance of P less than 10^{-20}. The average map density in this region was approximately 1 SNP per 30 kb. Newly discovered loci will be tested in a second population.

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Analysis of Neuropilin-1 as a Candidate Gene for Diabetic Nephropathy. *Y. Wang[1], M. Slaughter[1], J.R. Schelling[2,3], J.R. Sedor[2,3], S.K. Iyengar[1].* 1) Epidemiology & Biostatistics, Case Western Reserve University, Cleveland OH; 2) Department of Medicine, Case Western Reserve University,Cleveland OH; 3) Rammelkamp Center for Research and Education, MetroHealth Medical Center, Cleveland OH.

Diabetic nephropathy (DN) is a multifactorial disease with strong evidence for a genetic cause of disease pathophysiology. Interval mapping in rat strains susceptible to glomerulosclerosis has demonstrated that multiple loci (Rf1-Rf5) contribute to the renal failure phenotype. Previously, we showed that the Rf5 orthologous region on human 10p was linked to DN. Bioinformatic mining revealed that the Neuropilin1 gene (NRP1) is within 1cM interval of the 10p linkage peak. NRP1 is a co-receptor for vascular endothelial growth factor (VEGF) and Semaphorin ligands. Kidney glomerulus-specific deletion or overexpression of VEGF causes glomerular disease in mice. NRP1 is also expressed in kidney, but transgenic mice with overexpression or targeted deletion of NRP1 are embryonic lethal prior to kidney development. Hence, the role of NRP1 in the kidney is not well understood. Since glomerulosclerosis is a hallmark of DN, three SNPs were identified in NRP1 and genotyped in 74 European American (EA) and 79 African American (AA) diabetic sib pairs phenotyped for the presence or absence of DN. In our analysis, the SNPs were coded as covariates in three different genetic models: additive, dominant, and recessive. A Haseman-Elston regression linkage analysis was performed using the S.A.G.E. program SIBPAL. Based on the sibling data, we discovered that when SNPs at NRP1 were added as covariates, the previously identified linkage signal in the EA population lost significance (P-value increased from 0.00565 to 0.0482), while we observed a small increase in the linkage signal for the AA (P-value decreased from 0.650 to 0.428). When a test of family-based association between these SNPs and the DN trait was performed with ASSOC, we observed no association with any of the SNPs in either the EA or AA populations. Our conclusion is that NRP1 is in close linkage disequilibrium with a gene for DN, but is not the causative gene in this population.

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The Arg16Gly polymorphism in beta2-adrenoreceptor is associated with severe obesity in Pima Indian women. *T. Guo, Y. Muller, L. Ma, R. Valenzuela, R. Hanson, S. Kobes, C. Bogardus, L. Baier.* Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 4212 N. 16th Street, Phoenix, AZ 85016, USA.

The beta-2 adrenoceptor (ADRB2) is a major lipolytic receptor in human fat cells and may be of major importance for obesity and energy expenditure. To investigate the impact of single nucleotide polymorphsms (SNPs) in the *ADRB2* gene on obesity, we sequenced 83 extremely obese Pima Indians (BMI 50 kg/m2). A total of 10 common SNPs were found. Four SNPs were located in the exonic region, of which one was a functional missense substitution (Arg16Gly, rs1042713). Four SNPs were located in the 5' promoter region, and two in the 3' flanking region. A Gln27Glu, reported to be associated with obesity in most populations was not found to be polymorphic in Pima Indians. Eight of the 10 SNPs were successfully genotyped in 489 full-heritage, non-first-degree-related adult Pimas (362 severely obese and 127 nondiabetic-nonobese subjects) for association analysis. Analysis of stratification by gender showed 7 SNPs, including Arg16Gly, had nearly complete allelic concordance (r^2=0.78-0.94), and were significantly associated with obesity only in females (recessive model p= 0.0046-0.0175). Our data suggest that ADRB2 may influence body weight through a sex-specific mechanism, and our results are in accordance with results which showed that Gln27Glu, a polymorphism in the same haplotype block as Arg16Gly in Caucasians, to be associated with obesity in women only from Sweden, Spain and Japan.

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Lack of association between serotonin transporter gene and borderline personality disorder: a preliminary study. *X. Ni[1], K. Chan[2], N. Bulgin[1], T. Sicard[1], S. McMain[2], J. Kennedy[1].* 1) Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) Dialectical Behavior Therapy Clinic, Centre for Addiction and Mental Health, Toronto, Canada.

Borderline personality disorder (BPD) is a chronic, disabling, and high-risk mental disorder characterized by a pervasive pattern of instability in regulation of emotion, interpersonal relationships, self-image, and impulse control beginning in early adulthood. BPD affects about 1%-2% of the general population and has high mortality rate as a result of suicide and impulsive behaviour. Multiple lines of evidence suggest that serotonin transporter gene (5-HTT) plays an important role in suicide, impulsive behaviour, and emotional liability. To test for an association between 5-HTT and BPD, we genotyped two polymorphisms in 5-HTT, 5-HTTLPR and VNTR, in 63 Caucasian patients with BPD and 263 Caucasian healthy controls. The program COCAPHASE was used to compare allele frequencies between cases and controls and to perform haplotype analysis. There were no significant differences of allele and genotype frequencies of the 5-HTTLPR and VNTR markers between BPD patients and healthy controls ($p>0.05$). There was also a lack of association based on haplotype analysis. Our results suggest that serotonin transporter gene may not play a role in BPD. Naturally, the small sample of BPD patients may have limited our findings. The study of a large sample of BPD patients is warranted to substantiate these preliminary findings. This work is supported by Quick Action Small Grant Program of the Borderline Personality Disorder Research Foundation (BPDRF).

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Nicotine dependence is associated with the serotonin transporter gene. *S.L. Santangelo[1,2,3], G.D. Papandonatos[4], J.M. McCaffery[5], M. Lyons[2,6], M.T. Tsuang[2,3,7], R. Niaura[8], S. Buka[3,9].* 1) Psychiat & Neurodevlop Genetics, Ctr for Human Genetic Resrch, Mass General Hosp, Boston, MA; 2) Dept Psychiat, Harvard Medical School, Boston, MA; 3) Dept Epidemiology, Harvard School of Public Health; 4) Center for Statistical Sciences, Dept Community Health, Brown Medical School, Providence RI; 5) Ctrs for Behavioral and Preventive Medicine, Dept Psych & Human Behav, Brown Medical School & Miriam Hosp, Providence, RI; 6) Dept Psychology, Boston University, Boston, MA; 7) Institute of Behav Genomics, UC San Diego, San Diego, CA; 8) Ctr for Transdiscip Resrch, Dept Psych & Human Behavior, Brown Medical Schl & Butler Hosp, Providence RI; 9) Dept Society, Human Develop & Health, Harvard School of Public Health, Boston MA.

Most candidate gene studies of smoking phenotypes have investigated only one or two specific mutations, functional polymorphisms, or SNPs. With such sparse coverage, it is difficult to know what if any inferences should be drawn from negative results of such studies. With the recent availability of large numbers of SNPs in public databases, it is now possible to fully characterize a candidate gene by saturating it with SNPs at high density. Recently, we undertook such an investigation of the serotonin transporter gene (SLC6A4). A case-control association study was conducted to test for association of SLC6A4 with DSM-IV nicotine dependence. The genotyped sample was comprised of 443 unrelated individuals, of whom 211 were nicotine dependent. SLC6A4 spans 31 kb on chromosome 17q. Sixteen SNP markers selected from public databases were genotyped across 96 kb, including 25 kb past the 3 and 5 ends of the gene, for an average density of 6 kb. The 11/16 markers retained after data cleaning fell into three blocks of linkage disequilibrium. We identified a 5 SNP haplotype in block 1 with a frequency of .33 that was nominally significantly associated with nicotine dependence. This was accounted for by two common SNPs in strong LD with one another, with frequencies of .52 and .51. A Cochran-Armitage test for trend in allele frequencies indicated both SNPs were significantly associated with nicotine dependence, with p-values of 0.018 and 0.008.

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Psychosis and Mania: a 440-SNP screen of 65 candidate genes in Ashkenazi Jewish schizophrenia and bipolar 1 case/parent trios using two clinical conditions. *V.K. Lasseter[1], M.D. Fallin[2], G. Nestadt[1], P.S. Wolyniec[1], J.A. McGrath[1], D. Avramopoulos[1], N. Cheng[1], D. Valle[4,5], K.Y. Liang[3], A.E. Pulver[1,2].* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health; 3) Department of Biostatistics, The Johns Hopkins Bloomberg School of Public Health; 4) McKusick-Nathans Institute, The Johns Hopkins School of Medicine; 5) The Howard Hughes Institute, The Johns Hopkins School of Medicine.

Previously, we completed a SNP screen of 440 SNPs in 65 candidate genes for either bipolar 1 disorder or schizophrenia using Ashkenazi Jewish case/parent trios. The use of narrow phenotypes and a relatively genetically homogeneous population were strategies selected to increase power and the likelihood of success in identifying predisposing loci for these genetically complex psychiatric disorders. An overlap in genetic susceptibility for the two disorders has been hypothesized based on co-aggregation of familial risks and symptoms. From the sample of 323 bipolar 1 trios and the second sample of 274 schizophrenia trios, significant evidence (empirical $p<.01$) was previously shown for DAO, GRM3, GRM4, GRIN2B, IL2RB, and TUBA8 for bipolar 1 disorder and DPYSL2, DTNBP1, G30/G72, GRID1, and GRM4 for schizophrenia. We have now conducted exploratory genotype TDT association analyses (both single SNP and haplotype-based) using two new phenotypes based on consensus ratings of symptom-level data (manic episodes and psychosis) in these same Ashkenazi Jewish trios. For the mania phenotype (423 trios), we find significant ($p<.01$) evidence for 3 genes (KIF13A, BZRP, TUBA8). Similarly for the psychosis phenotype (494 trios), 6 candidate genes (KIF13A, SCA1, GRM4, ADRA1A, NOS1, and TPP2) had significant associations. These analyses address issues in heterogeneity and overlap in these severe psychiatric disorders and may be helpful in prioritizing future studies for other investigators.

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Refinement of the chromosome 5q31 risk haplotype in inflammatory bowel disease. *S.A. Fisher[1], J. Hampe[2], M.J. Daly[3], C. Onnie[1], S. Purcell[3], C.M. Lewis[1], S. Schreiber[2], J.D. Rioux[3], C.G. Mathew[1].* 1) Department of Medical and Molecular Genetics, King's College London, Guy's, King's and St Thomas' School of Medicine, London, United Kingdom; 2) Department of General Internal Medicine, Christian-Albrechts-Universitat, Kiel, Germany; 3) Whitehead Institute of Technology, Center for Genome Research, Massachusetts, USA.

Strong association of a common haplotype spanning 250 kb on chromosome 5q31 with Crohns disease (CD) has been widely replicated. It has been proposed that two functional variants within the *SLC22A4* and *SLC22A5* genes at this locus contribute directly to CD susceptibility. However, extensive linkage disequilibrium across the region has complicated efforts to distinguish causal variants from the strong association of the general risk haplotype.

We have genotyped the *SLC22A4* and *SLC22A5* variants (L503F, G-207C) and four other single nucleotide polymorphisms (SNPs) from the risk haplotype (IGR2063, IGRX100a, IGR2230, IGR3236) in a total of 1,200 cases and controls from four populations of European origin. A haplotype regression based approach has been used to test for conditional association and to dissect the contribution of individual SNPs or haplotypes to disease risk.

The results show that the C2063G variant, rather than L503F or G-207C, provides the strongest single SNP association and that, conditional on a single L503F SNP effect, a haplotype effect with C2063G significantly improved the fit of the model. We conclude that variants in the *SLC22A4* and *SLC22A5* genes are not sufficient to explain the disease risk at this locus. Resolution of the identity of the causative susceptibility gene or mutations will require very large association studies and may not be possible by genetic studies alone.

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Extension of the association probability to a quantitative trait. *W. Zhang, N. Maniatis, S. Rodriguez, I.N.M. Day, A. Collins, N.E. Morton.* Human Genetics Div, Univ Southampton, Southampton, United Kingdom.

The association probability was developed for haplotypes in case-control studies, extended to the transmission disequilibrium test, derived from the Malecot model and shown to be more efficient than alternative metrics to describe linkage disequilibrium (LD, Morton et al. 2001). From this foundation was extended to diplotypes and used to create the first LD maps. Recently a further extension was made to association mapping for affection status through the metric z = , where is the attributable risk in diplotypes. In the example of CYP2D6 data, no other model had performed as well in association mapping as the and z metrics, which are most powerful when LD is expressed in LD units (LDU, Maniatis et al. 2004). Here we present the theory and application of a quantitative trait association mapping using the z metric. The sample can be random, case-control, or case-hypernormal control. A quantitative trait is grouped into m classes and the class variable is expected to be robust to deviations from normality. Data with estimated z are fitted to the Malecot model and composite likelihood, 2 testing association obtained from the hierarchical models and location of the associated gene estimated. The method has been verified in the CYP2D6 data, where m = 2. We also applied the method to the IGF2 data comprising 2743 random samples from a UK multicentre study of cardiovascular risk. Weight, lean body mass (LBM), and the first principal component of these variables with body mass index (BMI) were analyzed. Preliminary results indicate that disease associated regions can be fine-mapped with high power by this approach. References: Morton NE, Zhang W, Tailon-Miller P, Ennis S, Kwok PY, Collins A (2001) The optimal measure of allelic association. Proc Natl Acad Sci USA 98: 5217-5221. Maniatis N, Morton NE, Gibson J, Xu CF, Hosking LK, Collins A (2004) The optimal measure of linkage disequilibrium reduces error in association mapping of affection status. Hum Mol Genet 14: 145-153.

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Disentangling linkage disequilibrium and linkage from dense SNP trio data. *G. Clarke, L. Cardon.* Wellcome Trust Center for Human Genetics, University of Oxford, Oxford, United Kingdom.

Parent-offspring trios are widely collected as part of disease gene mapping studies, and also being extensively genotyped as part of the International HapMap Project. With dense maps of markers on trios, the effects of LD and linkage can be separated allowing estimation of recombination rates in a model-free setting. Here we define a model-free multipoint method based on dense sequence polymorphism data from parent-offspring trios to estimate inter-marker recombination rates. We use simulations to show that this method has up to 92% power to detect recombination hotspots of intensity 25 times background over a region of size 10kb typed at a density 1 marker per 2.5kb and almost 100% power to detect large hotspots of intensity >125 times background over regions of size 10kb typed with just 1 marker per 5kb (=0.05). We found strong agreement at megabase scales between estimates from our method applied to HapMap trio data and estimates from the genetic map. At finer scales, using CEPH pedigree data across a 10Mb region of chromosome 20, a comparison of population recombination rate estimates obtained from our method with estimates obtained using a coalescent based approximate-likelihood method implemented in PHASE 2.0 shows detection of the same coldspots and most hotspots: The Spearman rank correlation between these estimates is 0.58 ($p< 2.2^{-16}$).

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Fine mapping candidate genes in cleft lip loci at 1p22-p33, 3q26-q28, 15q12-q14 and 17q12-31. *H. Mishima[1], L.M. Moreno[1], T. Busch[1], M. Arcos-B.[8], C. Velencia[3], A. Hing[4], E. Lammer[5], M. Jones[6], N. Robin[7], B.S. Maher[2], M.E. Cooper[2], T. Goldstein McHenry[2], A. Mach[1], M.L. Marazita[2], A.C. Lidral[1].* 1) U. of Iowa, Iowa City, IA; 2) U. of Pittsburgh, PA; 3) U. of Antioquia, Medellin, Colombia; 4) Children's Hosp., Seattle, WA; 5) Children's Hosp., Oakland, CA; 6) Children's Hosp., San Diego, CA; 7) U. of Alabama, AL; 8) NIH, Bethesda, MD.

Recent candidate gene and genome scan studies from Colombian and Ohio populations indicated that loci at 1p22-p33, 3q26-q28, 15q12-q14 and 17q12-31 met the criteria for follow up studies (LOD>1.75 or p<0.0023) (Rao and Gu 2001). Molecular data and animal models support candidate genes in these loci including *LHX8*, *FOXD3*, and *TGFBR3* (1p22-p33); *TP63* and *EPHB3* (3q26-q28); *GABRB3* (15q12-q13) and *WNT3* and *WNT9B* (17q12-31). The purpose of this study was to fine map these loci in an extended sample. 32 SNPs were genotyped in 78 extended families and 85 trios from Colombia and 28 extended families and 99 trios from USA. Parametric, nonparametric and association analyses were conducted. Results showed population differences with 1p22-p33 being positive in the USA data, while 3q26-28 being positive only in the Colombian data. *FOXD3* and *ROR1* genes at 1p22-p33 do not appear to increase risk for NSCL/P. However, markers within 102-126 cM at 1p22-p33 were significant (p<0.01) with the most significant results centered at 114cM (multipoint NPL p=0.00003). *EPHB3* and *TP63* genes at 3q26-28 showed positive LD in the Colombian data (p=0.058 and p=0.02, respectively) but not in the USA data. Markers for *GABRB3* at 15q12 revealed weak LD in both populations. Finally LD was also observed at 17q12-31 from SNPs rs1999533 (p=0.017, 69cM; *NSF*) and rs70602 (p=0.024, 69cM; *WNT3*) in 292 Colombian nuclear trios and also, significant LD was observed for the entire data set USA and Colombia (521 nuclear trios) with the SNP rs1530364 (p=0.014, 65cM; *WNT9B*). In conclusion, results obtained confirm the presence of NSCL/P susceptibility loci in 1p22-p33 in the US data, 3q26-q28 in the Colombian data and 17q12-31 for both populations underscoring the population heterogeneity that characterizes this trait. Supported by NIH R01DE14677, K02DE015291, P60DE13076; MOD #6-FY01-616.

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A Powerful and Robust Test of Transmission Disequilibrium for Quantitative Traits in General Pedigrees. *G. Diao, D.Y. Lin.* Dept Biostatistics, Univ North Carolina, Chapel Hill, NC.

Association mapping based on family studies can identify genes that influence complex quantitative traits while providing protection against population stratification. Among all the family-based tests of association, the one based on the variance-components model (Abecasis et al. 2000, Am. J. Hum. Genet. 66, 279-292) is the most flexible and most powerful. This test, however, relies heavily on the normality of the quantitative trait. Nonnormality can cause inflated type I error and reduced power. In most situations, the problems cannot be overcome by permutation. Although it is desirable to transform the trait values to achieve normality, it is difficult to specify the correct transformation, especially when there exist outlying trait values. We propose a novel extension of the variance- components model by allowing a completely arbitrary transformation function for the trait values, and present an efficient likelihood-based test for genetic association. Extensive simulation studies show that the proposed method provides accurate control of the type I error and is substantially more powerful than the existing methods. The advantages of our method are demonstrated through an application to the Collaborative Study on the Genetics of Alcoholism.

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Towards a systematic screen of cytoplasmic dynein-dynactin genes and other candidates for association with motor neuron degeneration disorders by tagging SNP analysis. *P.R. Shah[1], A. Ahmad-Annuar[1], K.R. Ahmadi[2], N. Soranzo[2], C. Russ[3], P.C. Sapp[4], D. Kasperaviciute[1], H.R. Horvitz[4], R.H. Brown Jr.[3], D.B. Goldstein[2], E.M.C. Fisher[1].*
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The cytoplasmic dynein-dynactin complex, a molecular motor responsible for retrograde axonal transport, has been implicated in the aetiology of motor neuron degeneration in both mouse models and humans. For example, mutation of the human dynactin subunit *DCTN1* is a risk factor for familial lower motor neuron disease (Puls et al. Nat Genet 2003; 33:455) and sporadic amyotrophic lateral sclerosis (SALS) (Munch et al. Neurology 2004; 63:724). Consequently, as genes of this complex and other candidates are of interest as causal/susceptibility loci for motor neuron degeneration disorders, we have developed a strategy to economically represent and interrogate common variation across each gene for an association with disease. We have determined haplotype and linkage disequilibrium patterns for each gene of interest in northern Europeans, based on single nucleotide polymorphism (SNP) genotype data. We identified SNPs capable of tagging known/unknown variation (tSNPs) with an association threshold (r^2) 0.85 and assessed their performance using a SNP-dropping procedure. We genotyped tSNPs in 261 North American SALS cases and 225 matched controls to test for association with disease.

To date, we have analysed tSNPs in several genes and completed studies for both cytoplasmic dynein 1 heavy chain 1 (*DYNC1H1*) and intermediate chain 1 (*DYNC1I1*). For *DYNC1H1*, we found that two tSNPs (rs2251644 and rs941793) were sufficient to tag the majority of haplotypic variation at $r^2$0.85 but found no significant association with disease (p=0.538 and p=0.204 respectively, tSNP haplotype p=0.956). In addition, 9 tSNPs were sufficient to tag *DYNC1I* and no significant association with disease status was seen (p>0.08). We are currently investigating additional genes of the complex.

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A Genome Variation Server for linkage disequilibrium analysis and tagSNP selection using genotype data from dbSNP. *M.J. Rieder, Q. Yi, P.D. Robertson, E. Calhoun, D.A. Nickerson.* Dept Genome Sciences, University of Washington, Seattle, WA 98195.

Selecting informative single nucleotide polymorphism (SNP) markers for genotype-phenotype studies requires genotype data from many individuals to assess the linkage disequilibrium (LD) between SNPs. The number of SNPs with genotype data submitted over the past year to dbSNP has increased dramatically (approximately 2 million SNPs) due to the efforts of the International HapMap Project and from genotypes generated by Perlegen (1.58 million SNPs across three populations). To provide a tool for rapid querying, data integration, and analysis of the genotype data in dbSNP, we have designed a web-accessible Genome Variation Server (GVS). This GVS allows users to query all SNPs with genotype data in the dbSNP database according to eithergenome coordinates, HUGO gene name, Entrez GeneID, or reference SNP identification number (dbSNP rs number). Each query returns the number of SNPs and genotypes by population (e.g. European, African-American, Asian, etc). Additionally, specific population data can be queried to extract and display genotype data in both text and graphical formats such as the visual genotype, a color-coded array of sample identifiers and their corresponding genotypes. Interactive queries, to select tagSNP sets based on user-defined thresholds forr minor allele frequency cutoffs and LD levels (using the r^2 LD measure), are used to generate summary text output and display LD plots graphically. GVS allows a simplified method for rapid retrieval, analysis and selection of customized tagSNP panels in specific populations.

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Linkage Disequilibrium Mapping With Single Feature Polymorphisms. *S. Kim[1], K. Zhao[1], R. Jiang[1], J. Molitor[3], J. Borevitz[2], M. Nordborg[1], P. Marjoram[2].* 1) Department of Biological Sciences, University of Southern California, Los Angeles, CA; 2) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 3) Department of Preventive Medicine, University of Southern California, Los Angeles, CA.

Single Feature Polymorphisms (SFP) are polymorphisms resulting from the use of high-density oligonucleotide tiling arrays for genotyping. For any feature (typically 25bp) on the array, any sequence differences between a particular chromosome and the reference genome will be revealed through a difference in hybridization intensities. The data are thus of the binary form same or different with respect to the reference genome. Because two chromosomes that both differ from the reference genome need not be identical and sensitivity and specificity are not identical, the data can be viewed as noisy SNP data with strange asymmetric errors. The advantage of SFPs over SNPs is their low cost. Here we examine the feasibility of using SFPs for linkage disequilibrium (LD) mapping. We compare the performance of tag SNPs to that of SFPs on simulated data with 2 different haplotype-based LD mapping methods. Our general conclusion is that for a given cost, SFPs will often have more power than SNPs as the former make up for quality with quantity.

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The first genome wide linkage disequilibrium map. *W. Tapper, A. Collins, J. Gibson, N. Maniatis, S. Ennis, N.E. Morton.* Human Genetics, University of Southampton, Southampton, SO166YD, UK.

Two genetic maps describe patterns of recombination and can be used to identify genes affecting a particular phenotype, recombinogenic sequences, and evidence of natural selection. Recombination is measured in morgans over a single generation in a linkage map, but may cover thousands of generations in a linkage disequilibrium (LD) map measured in LD units (LDU). The HapMap Project has led to a huge increase in the genotyping of single nucleotide polymorphisms (SNPs) that are the main source of LD information. We have used a subset of the phase 1 HapMap data consisting of 2,885,936 SNPs from CEPH (CEU), Japanese (JPT), Chinese (HCB), and Yoruban (YRI) populations, to create the first genome wide LD map which covers 99.7% (2,934 Mb) of the euchromatin. Recombination accounts for 97.1% of the LDU variance in chromosome arms and 95.2% in their deciles. The Malecot model predicts that the ratio of corresponding distances in LDU and Morgans estimates the effective bottleneck time t in generations which is constant between chromosomes. The LD maps estimate t as 1472, 1483, 1648, and 2073 generations for CEU, JPT, HCB, and YRI respectively implying bottleneck times of between ~36,800 and ~51,825 years, assuming 25 years per generation. Presumably, this low estimate reflects the partly cumulative effect of bottlenecks since the out-of-Africa migration. Deeper analysis shows significant deviations in t between chromosome arms due to physical size, SNP density and holes in the LD map. These differences are the result of (1) the linkage map employing the Kosambi function which exagerates Morgan length for all chromosomes especially shorter ones; (2) the non-pseudoautosomal region of the X chromosome being subject to hemizygous selection; and (3) low SNP density in intervals of high recombination. These genome wide LD maps are available from our LD database (http:/cedar.genetics.soton.ac.uk/public_html/LDB2000/release.html) as integrated maps that can be viewed as tables or graphs to determine the pattern of LD in any genomic region. Other options on this site allow for identification of recombination hotspots, population comparisons and SNP selection based on LDUs.

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Tag SNP selection for Finnish individuals based on the CEPH Utah HapMap database. *C.J. Willer[1], L.J. Scott[1], L.L. Bonnycastle[2], A.U. Jackson[1], P. Chines[2], R. Pruim[1,3], C.W. Bark[4], Y-Y. Tsai[4], E.W. Pugh[4], K.F. Doheny[4], L. Kinnunen[5], T.T. Valle[5], R.N. Bergman[6], J. Tuomilehto[5,7,8], F.S. Collins[2], M. Boehnke[1].* 1) Dept of Biostatistics, Univ Michigan; 2) Genome Technology Branch, National Human Genome Research Institute; 3) Dept of Mathematics and Statistics, Calvin College; 4) Center for Inherited Disease Research, School of Medicine, Johns Hopkins University; 5) Diabetes and Genetic Epidemiology Unit, Dept of Epidemiology and Health Promotion, National Public Health Institute, Finland; 6) Dept of Physiology and Biophysics, Keck School of Medicine, Univ of Southern California; 7) Department of Public Health, University of Helsinki, Finland; 8) South Ostrobothnia Central Hosp, Seinäjoki, Finland.

The Finland-United States Investigation of NIDDM Genetics (FUSION) study has sought to evaluate tag SNPs selected for the purpose of association testing in a region of linkage. In an 18 Mb region of chromosome 14, we selected SNPs with minor allele frequency 5% from the CEPH Utah HapMap database (May 2004) by applying a pairwise r^2 criterion of .8 and prioritizing selection of nonsynonymous SNPs and those with higher Illumina design scores. Genotypes for 1,425 Finnish samples at 956 SNPs were generated at CIDR. After matching of the reference allele, 90% of SNPs had an allele frequency difference .10 between the CEPH Utah and Finnish samples and 99% of SNPs had an allele frequency difference .15. The t-test revealed 7.7% of the SNPs had a significant difference (p .01) between allele frequencies in the CEPH Utah and Finnish samples. However, after examining pairs of adjacent SNPs, we found that the absolute difference between the r^2 estimates in the Finnish and CEPH samples was .10 in 76% of SNP pairs and .15 in 87% of SNP pairs. Haplotype frequency estimates for 180 bins of 3-20 SNPs also showed similarities between the Finnish and CEPH Utah samples; 94% and 98% of haplotypes had frequency differences .10 and .15, respectively. We conclude that use of the CEPH Utah linkage disequilibrium data allowed for efficient SNP selection in our Finnish sample.

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Linkage disequilibrium and haplotype study at the Xq13-21 region in six Native American populations. *S. Wang[1], D. Labuda[2], G. Bedoya[3], A. Ruiz-Linares[1,3].* 1) Wolfson House, The Galton laboratory, Department of Biology, University College London, London, United Kingdom; 2) Université de Montreal, Montreal; 3) Laboratorio de Genética Molecular, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.

Great interest exists in assessing patterns of linkage disequilibrium (LD) in human populations as these will impact on our ability to identify genes through association analyses. A region of the world that has been scantly examined in LD surveys is the American continent. Here we examined LD in six Native American populations at a region of the X chromosome (Xq13-21) that has been extensively studied in Europe and Asia. We typed seven microsatellite markers covering this 4cM region in six Native American populations (five from Colombia and one from Canada). Compared to other populations, Native Americans show low diversity and high LD. Similar levels of LD were observed in Native Americans as in certain European and Asian isolates, but with lower diversity in the Americas. The haplotype frequency distribution at the two markers in strongest LD shows that three haplotypes that are rare in Asia or Europe have a widespread distribution in the Americas, while the two most common haplotypes in Asia and Europe were not observed in the Americas. These results are consistent with strong drift in Native American populations and illustrate the potential of X-chromosome studies for probing the evolutionary history of the Americas. The low genetic diversity and high LD of Native American populations should facilitate association mapping of common diseases in these populations.

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Association of IL4Ra gene with severity of lung disease in Cystic Fibrosis. *F. Belpinati[1], G. Malerba[1], L. Xumerle[1], C. Bombieri[1], R. Galavotti[1], C. Castellani[2], B.M. Assael[2], P.F. Pignatti[1].* 1) Sect Biol & Genetics, DMIBG, Univ Verona, Verona, Italy; 2) Cystic Fibrosis Veneto Regional Centre, Hospital of Verona, Italy.

Cystic Fibrosis is an autosomal recessive disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Chronic lung disease is the most serious clinical expression of CF. The course of the lung disease is highly variable; this variability is explained only partially by allelic heterogeneity at the CFTR gene. The severity of pulmonary manifestations in CF may be correlated with other genetic factors. We report the study of three polymorphisms of the IL4Ra (IL4-receptor alpha) gene. This gene is involved in asthma and atopy and may represent a possible modifier of pulmonary disease in CF. Eigthy-three family trios were collected through a CF patient attending the Veneto Regional CF Centre of Verona. All the 83 patients were severe/severe CFTR mutation carriers and were clinically evaluated for respiratory parameters, gastrointestinal and nutritional status parameters, and other clinical variables. The patients and their parents were investigated for three functional polymorphisms (I50V, S503P and Q576R in exons 3 and 9) of the Il4Ra gene. The transmission disequilibrium test showed a significant association between Q576R (p=0.014) or S503P (p=0.035) polymorphisms with FEV1 values in the patients. The Q576R and S503P polymorphisms showed to be in strong linkage disequilibrium (D'=0.98, r2=0.79). The I50V was not in linkage disequilibrium with any of the other 2 polymorphisms. In conclusion, these data suggest that the IL4Ra gene might be a modifier gene for FEV1 variability in CF, mediating lung inflammatory processes.

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Linkage Analysis for Bilaterial Perisylvian Polymicrogyria: a new locus mapped on Xq27-q28. *N. Santos[1], I. L. Brandão[1], R. Secolin[1], M. S. Silva[1], F. Cendes[2], M. M. Guerreiro[2], I. Lopes-Cendes[1].* 1) Dept Medical Genetics, UNICAMP, Campinas, SP, Brazil; 2) Dept Neurology, UNICAMP, Campinas, SP Brazil.

Introduction: Recently, familial forms of bilaterial perisylvian polymicrogyria (BPP) have been described and a candidate locus was mapped on chromosome (ch) Xq28, distal do marker DXS8103. The objective of this study was to describe clinical characteristics and to perform linkage analysis in 3 families segregating bilateral perisylvian polymicrogyria (BPP).

Methods: We studied 3 unrelated families segregating BPP. A total of 26 individuals, including 14 patients were evaluated in this study. All family members were examined by a neurologist and subjected to high resolution volumetric MRI scans with multiplanar reconstruction. Family members were genotyped for 14 polymorphic dinucleotide repeat markers: DXS1192, DXS1227, DXS8106, DXS8084, DXS8043, DXS8028, DXS1200, DXS998, DXS8091, DXS8086, DXS8069, DXS8103, DXS1073 and DXS8087, flanking a 42.3 cM interval on ch Xq27-q28. Two-point and multipoint linkage analysis was performed using the LINKAGE package.

Results: Clinical spectrum ranged from normal to mild neurological dysfunction, mainly pseudobulbar paresis, such as poor articulation and poor tongue movements. All 14 patients had BPP confirmed by MRI, in different anatomical distributions: 9 patients had only BP, 3 had posterior parietal BPP and 2 had frontoparietal BPP. Two-point linkage analysis yield a Zmax = 3.10 at = 0.00 for DXS1227 for all 3 families combined. Multipoint and haplotype analysis pointed to a candidate interval within a 12 cM region between markers DSXS1227 and DXS8043.

Conclusions: Our results point to a different candidate region on ch Xq in familial forms of BPP. In addition, our data places the candidate interval to a 12 cM region mapped to Xq27-q28, a more centromeric location than previously reported.

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Mapping of an autosomal dominant cataract (ADC) Chilean family to a novel locus. *L. Richter[1], D. Burch[1], F.R. Barría von Bischhoffshausen[2], P. Flodman[3], M.A. Spence[3], J.B. Bateman[1].* 1) Department of Ophthalmology, Rocky Mountain Lions Eye Institute, University of Colorado, Aurora, CO; 2) Departamento de Oftalmología, Universidad de Concepcíon, Concepcíon, Chile; 3) Department of Pediatrics, University of California Irvine, Orange, CA.

Purpose: To map and identify the gene for autosomal dominant cataracts (ADC) in a large Chilean family.

Methods: ADC 54 is a four generation Chilean family consisting of 31 individuals with 13 individuals affected with cataracts. Clinically, affected individuals had cataracts with variable morphology (anterior polar, cortical, embryonal, one affected individual had microcornea). SIMLINK analysis was used to estimate the power to detect linkage in ADC 54. We screened the family with a panel of polymorphic DNA markers for known ADC loci using PCR amplifications performed separately for each primer set. The products were resolved on an ABI 373/3100 AVANT using Genemapper 3.5.1 software. Two point LOD scores were calculated using LIPED.

Results: For a tightly linked marker, we estimated that the maximum LOD score achieved over 1000 simulations would be 6.51. We calculated LOD scores between the ADC 54 locus and known ADC loci on chromosomes 1, 2, 3, 10, 11, 12, 13, 15, 16, 17, 19, 20, 21 and 22 and excluded all based on lack of evidence for linkage or co-segregation.

Conclusions: Using our ADC screening panel, we excluded linkage in this family with markers known to be linked to human ADC. This excludes all known ADC loci. Therefore, ADC 54 represents a novel ADC locus. We are currently running a genome-wide screen to identify the novel locus.

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The effect of linkage disequilibrium on multipoint linkage analysis. *D.F. Levinson[1], J.A. Knowles[2], J.B. Potash[3], R.R. Crowe[4], J.R. DePaulo[3], W.A. Scheftner[5], M.M. Weissman[2], P. Holmans[6].* 1) Dept Psychiatry, Univ Pennsylvania, Philadelphia, PA; 2) Columbia University and New York State Psychiatric Institute, New York, NY; 3) Johns Hopkins University, Baltimore, MD; 4) University of Iowa, Iowa City, IW; 5) Rush University Medical Center, Chicago, IL; 6) Biostatistics & Bioinformatics Unit, Wales College of Medicine, Cardiff, UK.

Marker-marker linkage disequilibrium can inflate multipoint linkage scores when parental genotypes are missing. This is an important problem to recognize and manage now that dense SNP marker sets are available. We studied this problem using both a novel simulation method and SNP data from the chromosome 15q candidate region for major depression (GenRED). Genotypes were simulated for 176 SNPs (Affymetrix chromosome 7 map, 60 Mb, 100 replicates, 116 sib-pair families) without linkage, using parental haplotypes inferred from COGA pedigrees (GAW14). Linkage was analyzed (ALLEGRO) with and without parental genotypes. Then, in depression pedigrees, linkage was analyzed using 6 microsatellites plus either 147 high-MAF SNPs (22 MB) or 115 SNPs (low-LD map, all marker-marker r-squared values <0.05). Intermarker distances were inferred from a framework map (CRIMAP). In simulated data, if parental genotypes were missing, linkage scores were inflated in regions of high marker-marker LD, or when marker-marker distances were inflated upward. In the GenRED data, a sharp peak was observed in a region of 15q with substantial LD. Using the low-LD map, the spurious peak was eliminated, while the linkage signal remained. Marker-marker LD can result in substantial inflation of linkage scores. This can be controlled by selecting markers with low pairwise LD for linkage analysis. Other approaches to correcting for LD should be tested on data with complex patterns of LD, which is often limited to or maximal between contiguous pairs of markers.

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Improvement of mapping accuracy by unifying linkage and association analyses. *X.-Y. Lou[1], J.Z. Ma[1], M.C.K. Yang[2], J. Zhu[3], P.-Y. Liu[4], H.-W. Deng[4], R.C. Elston[5], M.D. Li[1].* 1) Department of Psychiatry, UT Health Science Center, San Antonio, TX; 2) Department of Statistics, U of Florida, Gainesville, FL; 3) Department of Agronomy, Zhejiang University, Hangzhou, P.R. China; 4) Osteoporosis Research Center, Creighton University, Omaha, NE; 5) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

It is well known that pedigree/family data record information on the coexistence in founder haplotypes of alleles at nearby loci and the cotransmission from parent to offspring that reveal different, but complementary, profiles of the genetic architecture. Either conventional linkage analysis or family-based association tests (FBATs) capture only partial information, leading to inefficiency. In this article, we propose a likelihood-based approach that embeds both linkage and association analyses into a unified framework for general pedigree data. Relative to either linkage or association analysis, the proposed approach is expected to have greater estimation accuracy and power. Monte Carlo simulations support our theoretical expectations and demonstrate that our new methodology: (1) is more powerful than either FBATs or linkage analysis; and (2) can unbiasedly estimate genetic parameters regardless of whether association exists, thus remedying the bias and less precision of traditional linkage analysis in the presence of association. The new approach also holds the theoretical advantage that it can extract statistical information to the maximum extent and thereby improve mapping accuracy and power because it integrates multilocus population-based association study and pedigree-based linkage analysis into a coherent framework. Furthermore, our method is numerically stable and computationally efficient, as compared to existing parametric methods which use the simplex algorithm or Newton-type methods to maximize the high order multidimensional likelihood functions, and also offers the computation of Fisher's information matrix. Finally, we apply our methodology to a genetic study on bone mineral density (BMD) for the vitamin D receptor (VDR) gene and find that VDR is highly associated with the BMD at the one-third region of the wrist.

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Evidence for genetic loci influencing intraocular pressure: A Genome Wide Scan of the Beaver Dam Eye Study.

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Glaucoma is a leading cause of blindness in the world, and the identification of genes that contribute to this disease is essential. Elevated intraocular pressure (IOP) is a principal risk factor for primary open-angle glaucoma (POAG), and an intriguing quantitative trait that may strongly influence disease development. We performed a non-parametric genome-wide linkage analysis of 1019 sibling pairs with quantitative measurements for IOP. The sib pairs were ascertained through a population-based cohort, the Beaver Dam Eye Study. Higher IOP measurement between the eyes was used as a continuous trait, and treatment with drops, systolic BP, sex and age were covariates adjusted prior to analysis. IOP was normally distributed and remained normal after adjustment for covariates. We performed single point and multipoint linkage analysis using the modified Haseman-Elston regression models in SIBPAL (S.A.G.E. v4.5). We used the option that allows for non-independence of sib pairs and uses a weighted combination of squared trait difference and squared mean corrected trait sum. For each marker, pvalues were obtained using the asymptotic distribution of the likelihood-ratio test statistics. We also obtained empirical p values using Monte Carlo permutations with up to 1 million replicates. The GWS identified 3 linkage regions of interest, 1 of which reached suggestive evidence for linkage according to Lander & Krugylak thresholds in GWS. This novel linkage region had empirical p values of 2 x10-4 (singlepoint) and 5.1 x 10-5 (multipoint). These 3 regions are especially interesting since all 3 have been identified as potential linkage regions in GWS of hypertension or blood pressure. The results of this GWS scan provide evidence that a QTL may influence elevated intraocular pressure and that there may be multiple loci and heterogeneity for this trait.

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A Genomewide Linkage Study of Retinal Vessel Caliber in the Beaver Dam Eye Study. *C. Xing[1], B. Klein[3], R. Klein[3], G. Jun[1], K. Lee[3], S. Iyengar[1,2].* 1) Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Department of Ophthalmology, Case Western Reserve University, Cleveland, Ohio; 3) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, Wisconsin.

Retinal vessels can be observed non-invasively and provide a window to microvascular systems elsewhere in the body. It was believed that the retinal vessels are susceptible to changes in blood pressure, and that generalized retinal arteriolar narrowing represents structural changes resulting from persistent high blood pressure. However, recent studies also support an alternative hypothesis that generalized retinal arteriolar narrowing may precede hypertension, and may be genetically determined. We performed a genome wide model-free linkage scan by Haseman-Elston regression on the central retinal artery equivalents (CRAE) and central retinal vein equivalents (CRVE) using data from the Beaver Dam Eye Study (487 families, 812 sib pairs). Prior to linkage analyses, data were adjusted for significant covariates age age2, gender, smoking status, serum high density lipoprotein cholesterol for both traits (and diastolic blood pressure for CRAE). There were 7 regions on 6 chromosomes (1p36, 6p25, 6q14, 8q21, 11p15, 13q34, 14q21) showing linkage signals for either covariate -unadjusted or -adjusted CRVE, and were 7 regions on 5 chromosomes (3q28, 5q35, 7q21, 7q32, 11q14, 11q24, 17q11) showing linkage signals for either covariate -unadjusted or -adjusted CRAE, at the nominal multipoint significance level of 0.01. The best evidence for linkage was on 8q21 at marker D8S2324 with a multipoint p-value of 5.8 x 10-4, and 3q28 at marker D3S2418 with multipoint p-value of 8.7 x 10-7 for CRVE and CRAE, respectively. CRAE and CRVE shared linkage signals at 1p36, 6p25 and 7q21. In conclusion, retinal vessel caliber for arterioles and venules is dictated by both shared as well as distinct genetic components.

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A genome-wide scan of Familial Benign Recurrent Vertigo by use of a high-density single-nucleotide-polymorphism (SNP) genotyping assay: Suggestive linkage to 5p15, 15q21 and 22q12 with evidence of heterogeneity. *H. Lee[1], J.C Jen[2], H. Wang[5], C. Sabatti[1,5], R.W. Baloh[2,3], S.F. Nelson[1,4].* 1) Dept Human Genetics; 2) Dept Neurology; 3) Dept Surgery; 4) Dept Psychiatry and Biobehavioral Science, UCLA School of Medicine; 5) Dept Statistics, Univ California, Los Angeles, Los Angeles, CA.

Vertigo is a perception of movement, either of oneself or of the surround and it is caused by disturbances of the vestibular system from inner ear to brain pathways. Familial benign recurrent vertigo (BRV) is one of the most common reasons for referral to Neurotology Clinic, and many of these patients report a family history as well as an increased incidence of migrainous headaches. However, there is little understanding of the molecular basis of BRV, nor its possible molecular association with migraine, and there have been no prior systematic linkage studies in families with BRV. We present a genome-wide scan by both parametric (model-based) and non-parametric (model-free) analysis of 29 families with BRV showing strong association with co-morbid migraine. With the Affymetrix 10K SNP Mapping Assay, we could perform rapid, accurate and efficient genotyping across the whole genome at a mean intermarker distance of 210kb. Heterogeneity LOD (HLOD) score results show strong evidence of heterogeneity in the BRV population. We could exclude most of the genome as harboring a single gene causing the disease in half of the families in our sample set. Non-parametric analysis shows excess sharing among the affected members of the families. Three regions of the genome are indicated as being suggestively linked to BRV: Chromosome 22q12 by HLOD analysis (2.99), Chromosome 5p15 (2.85) and Chromosome 15q21 (1.9) by NPL. We conclude that BRV is a heterogeneous genetic disorder and that additional family and population based linkage and association studies will be needed to determine the causative alleles.

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Genome-wide linkage study for rate of change in blood pressure with longitudinal data in a Nigerian population.

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Most genome scans for blood pressure (BP) have been cross-sectional using phenotypic measurements at one time point. It is known that multiple longitudinal blood pressure measurements are more representative of a persons average or true blood pressure. In addition, rate of change in blood pressure over the study period can be estimated. Three sets of measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained every 2 years from 1998-2002 in families recruited from a rural area in west Nigeria. Genotypic data from 1,219 individuals in 286 families were included in this analysis. Linear mixed-effects models were utilized to obtain the estimates of random effect of rate of change of SBP and DBP for each individual adjusted for gender and BMI. Genome scans were performed on these derived phenotypes. The estimates of heritability for the rate of change of SBP and DBP are 33% and 35%, respectively. The strongest linkage evidence for rate of change of SBP was found at 10p14 region (marker D10S1412, LOD = 2.36). Likewise, the strongest linkage evidence for the rate of change of DBP (marker D10S1430, LOD = 1.96) was also found in this region.

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Autosomal genome scans for two clinical phenotypes (psychosis and manic episode) in multiplex Ashkenazi Jewish pedigrees with schizophrenia, schizoaffective disorder and/or bipolar disorder. *J.A. McGrath[1], V.K. Lasseter[1], M.D. Fallin[2], K.Y. Liang[3], N. Cheng[1], P.S. Wolyniec[1], D. Valle[4,5], D. Avramopoulos[1], P.L. Belmonte[2], A.E. Pulver[1,2].* 1) Department of Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD; 2) Department of Epidemiology, the Johns Hopkins Bloomberg School of Public Health; 3) Department of Biostatistics, the Johns Hopkins Bloomberg School of Public Health; 4) McKusick-Nathans Institute, The Johns Hopkins School of Medicine; 5) Howard Hughes Institute, The Johns Hopkins School of Medicine.

Previously we reported significant linkage to chromosome 10q22 for schizophrenia using 29 Ashkenazi multiplex pedigrees (Fallin et al., 2003) and suggestive evidence for chromosomes 1, 3, 11, and 18 using an independent sample of 22 multiplex bipolar 1 pedigrees (Fallin et al., 2004). Given potential clinical and genetic overlap for the diseases, we have identified clinical phenomena to serve as phenotypes (psychosis and manic episodes). For our multiplex Ashkenazi Jewish families with schizophrenia, schizoaffective disorder, and bipolar disorders, sixty-one families had at least two individuals who have experienced psychotic symptoms and thirty six families had at least two individuals who were reported to have had a manic episode. Linkage analyses of 390 autosomal markers (average 9 cM density) were completed (GeneHunter V2.1). Moderate support for chromosomes 1q (NPL=2.47, p=.005) and 10q (NPL=2.29, p=.01) were obtained for psychosis. For mania, we report moderate support for chromosome 11 (NPL=2.05, p=.02). Given the symptom/syndrome overlap between schizophrenia, schizoaffective disorder and bipolar disorders, evidence that these disorders may co-segregate in families and presumed genetic heterogeneity in both schizophrenia and bipolar 1 disorder, we conducted exploratory analyses using two phenotypes based on consensus ratings of symptom-level data (manic episodes and psychosis) in an Ashkenazi Jewish sample. These analyses may help in prioritizing for further study chromosomal regions related to syndromes that transcend current disorder classifications.

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Cleft lip genome scan reveals major loci, heterogeneity and genetic interactions. *L. Moreno[1], M. Arcos-B.[2], B.S. Maher[3], T. Goldstein McHenry[3], M.E. Cooper[3], C. Valencia-R.[4], K. Krahn[1], A. Hing[5], M. Moreno[1], H. Mishima[1], E.M. Lammer[6], M. Jones[7], N.H. Robin[8], M.L. Marazita[3], A.C. Lidral[1].* 1) University of Iowa, Iowa City, IA; 2) NIH, Bethesda, MD; 3) University of Pittsburgh, Pittsburgh, PA; 4) University of Antioquia, Medellín, Colombia; 5) University of Washington, Seattle, WA; 6) Children's Hospital Oakland Research Institute, Oakland, CA; 7) Children's Hospital, San Diego, CA; 8) University of Alabama, Birmingham, AL.

Nonsyndromic cleft lip and palate (NSCL/P) is a common birth defect with complex etiology. The purpose of this study is to identify regions containing susceptibility genes for (NSCL/P). A 10 cM genome scan was performed on 48 multiplex families from Colombia-SA and 9 multiplex families from Ohio, USA using genotypes generated by the Center for Inherited Disease Research (CIDR). Parametric, nonparametric and association analyses were performed. Also, conditional and covariate-based linkage methods were used to screen the Colombian families for genetic interactions and heterogeneity using both candidate gene and genome scan data. The Colombian families showed suggestive linkage at 3q26-q28, 9q22-q33, 14q24-q31, 15q11-q13.3 and 19q13 with the most significant findings at 9q22-q33 (Maximum LOD score (MLS)=2.94) and 3q27 (MLS=2.99). The addition of SNPs within 9q22-q33 candidate genes on 72 families revealed the most significant multi-point linkage at TGFBR1 (HLOD= 2.24). Suggestive linkage for the Ohio families was observed at loci in 3p14-p23, 9q22-q33, 12q24, 14q11-q22, 17q11-q21, 18q12 and significant linkage was observed at 1p22-p33 (MLS=3.52). Interactions were observed in the Colombian families between candidate gene markers in 9q22-q33 and 1p36.3 ($p = 0.00011$), 8q24.1 ($p= 0.000076$), and 11q22 ($p = 0.00011$). Furthermore, genetic heterogeneity was suggested since a subset of families showed linkage to 2q24.1-q32.1 and not 9q22-q33. In conclusion we have identified a major locus for NSCL/P at 9q22-q33 that may be modified by genes at other loci and another locus for CL/P maps to 2q24.1-q32.1. Supported by NIH P50-DE016215, NIH/NIDCR RO1DE14677, KO2DEO15291 and MOD Grant #6FY01-616.

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A novel X-linked locus for retinitis pigmentosa. *Z. Tong, Z. Yang, Y. Zhao, L. Xue, J. Barid, A. McGinnis, Y. Chen, E. Pearson, K. Zhang.* Department of Ophthalmology and Visual Science, Program in Human Molecular Biologoy and Genetics, Eccles Institute of Human Genetics, University of Utah, Salt lake city, UT. 84112.

PURPOSE: Retinitis pigmentosa (RP) is a heterogeneous group of inherited diseases which cause the progressive loss of night and peripheral vision. It affects over 1.5 million people worldwide. Linkage analyses indicate that there are at least five X-linked RP genes. We investigated a large Utah pedigree spanning 6 generations with 191 individuals with X-linked RP. METHODS: Peripheral blood was taken from fifty-three members, including 13 affected males and 15 carriers. Participants underwent ophthalmologic examination. Genotyping to X-linked RP loci (RP2, RP3, RP6, RP23 and RP24) were performed, and mutation screening of RP2 and RPGR was performed by PCR and direct DNA sequencing. RESULTS: Age of onset ranges from 5-8 years, visual acuity ranges from 20/25 in children to light perception in older adults. Linkage analysis and direct sequencing showed that no known loci/genes were associated with the phenotype in this kindred. CONCLUSION: Our results excluded all known X-linked loci for RP, and suggested a novel disease gene associated with X-linked RP. Linkage mapping is in progress. Identification of a new gene for X-linked RP will increase our understanding of RP and provide new therapeutic avenues.

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Back to genetics: increasing the power of tiny families for linkage analysis using haploid cell lines from an ALS family. *K.A. Dick[1, 2], J.C. Dalton[1, 2], M.B. Miller[3], J.W. Day[2, 4], L.P.W. Ranum[1, 2].* 1) Dept of Genet Cell Biol & Devel; 2) Inst of Hum Genet; 3) Dept of Epid & Comm Health; 4) Dept of Neurol, University of Minnesota, Minneapolis, MN.

The requirement of collecting families with many affected individuals is a major impediment to mapping novel disease genes. Identifying novel ALS genes is particularly problematic because the rapidly fatal disease course limits the ability to collect affected individuals. Over 16 yrs, we collected 12 ALS family members including 5 affected with a dominant, typical, adult-onset form of the disease. Based on family relationships only ~3% of the genome should be shared among the affected individuals, however, LOD score analysis is not effective in predicting these shared regions. To overcome these power limitations, we developed a novel mapping approach using haploid cell lines. Panels of haploid cell lines were generated for 8 individuals and an 800 marker genome scan performed on DNA from the haploid cells and diploid lymphocyte DNA. The chromosome separated cell lines enabled haplotypes to be directly defined along entire chromosomes, allowing common regions transmitted from a founder in generation I to be determined. Haploid mapping identified 8 regions (3.9% of the genome) shared among the 5 affected individuals and definitively excluded 87.7% of the genome as unshared. LOD score analyses excluded only 67.8% or 63.0% (LOD<-2 for FASTLINK and MERLIN, respectively), failed to identify any shared regions (LOD>3), and generated suggestive scores (most between 0.5-1.2) for areas that were both shared and definitively excluded by haploid analysis. These results enabled us to exclude previously reported ALS loci and coarsely map this novel gene to one of 8 regions spanning a total of ~117cM. The mapping and identification of additional disease genes will lead to a better understanding of gene function and disease mechanisms. Our haploid method will enable genetic investigations of a whole class of families with Mendelian disorders too small for classic linkage studies. In addition, haploid mapping provides a novel and independent approach to characterize and compare traditional linkage strategies.

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Inclusion of a Propensity Score as a Single Covariate in a Genome Wide Mega-Linkage Analysis Identifies New Significant Regions on 5p, 9p, 20p for Ulcerative Colitis and on 19q for Crohns Disease. *B.Q. Doan[1,2], C.E. Frangakis[3], J.E. Bailey-Wilson[2], R.H. Duerr[4], J.D. Rioux[4], M.S. Silverberg[4], H. Yang[4], S.R. Brant[4], P. Schumm[4], J.H. Cho[4], Y.Y. Shugart[1].* 1) Epidemiology, JHSPH, Baltimore, MD; 2) IDRB, NHGRI/NIH, Baltimore, MD; 3) Biostatistics, JHBSPH, Baltimore, MD; 4) NIDDK IBD Genetics Consortium.

A genome wide (GW) covariate-based linkage analysis was performed on a dataset (993 affected relative pairs) of five published scans for inflammatory bowel disease (IBD). Age at diagnosis, gender, and smoking status at diagnosis were included as covariates as they are known risk factors for disease onset. However, each covariate analyzed requires an additional parameter to be estimated in the linkage test which can reduce power or increase the type I error rate. A propensity score (PS) defined as the predicted conditional probability of being affected given the observed covariate data was considered to collapse multiple covariates into one, and was used to distinguish linked and unlinked subgroups where those with high scores can be interpreted as being less genetic. With no covariate data on unaffected individuals, the conventional PS values could not be computed. However, since IBD consists of two distinct subtypes, Crohns disease (CD) and ulcerative colitis (UC), a subtype-specific PS (predicted probability of having a specific subtype of IBD given the observed covariate data) was instead considered. Significance was determined by permutation-based p-values. With no covariates analyzed, the IBD3 locus on 6p yielded the greatest evidence of linkage ($p<0.005$) for IBD and CD, while the 2p locus was the most significant peak for UC ($p<0.005$). Incorporating smoking status increased power to detect the 5q locus for IBD, and using a PS based on age at diagnosis and gender identified new significant loci on 19q for CD, as well as 5p, 9p, and 20p for UC. These results suggest that covariates, such as a subtype-specific propensity score, can potentially increase power to detect linkage for a complex disease such as IBD, especially when multiple important covariates can be modeled to minimize heterogeneity within the pooled datasets.

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A linkage analysis test statistic which models relationship uncertainty. *A. Ray[1], D.E. Weeks[1,2].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA.

Linkage analysis methods assume that the stated familial relationships are correct. However, misspecified relationships can lead to reduced power for linkage. Possible solutions are to discard the erroneous data or to use the most likely alternative pedigree structures or to statistically model the uncertainty by weighting over the possibilities. Consider the situation where we have collected affected relative pair (ARP) data and carried out a genome-wide scan for linkage. In such a situation, we have two sources of information about relationships, the stated apparent relationship and the genome-wide marker data. Our method is an extension of the affected relative pair LOD score analysis approach of Cordell et al. (2000). In our extension, we statistically model any relationship uncertainty via weights, the weights being the conditional probability of a true relationship type given the apparent one and the genome-wide marker data. For an ARP with an apparent relationship, we consider several possible true relationship types. The estimates of the weights can be used to fit a likelihood model to the IBD sharing among ARPs. To assess our method and to compare it to the maximum likelihood statistic of Cordell et al. and a nonparametric linkage statistic from MERLIN, we are performing a simulation study. Each simulated dataset consists of 300 pedigrees with 660 affected apparently full sib pairs having several underlying true relationship types with marker data having 367 markers and a disease locus on chromosome 10 with a dominant reduced penetrance mode of inheritance. For each simulation, we also construct both the true relationship structure and the pedigree after discarding the individuals with erroneous relationships and obtain the MLS of our method, the MLS of Cordell et al. and the NPL statistic from MERLIN at each position in the genome. Preliminary results indicate our method perfoms well: Based on 1,650 replicates using genome-wide empirical 0.01 significance thresholds, power at 25 cM and 20 cM from the disease locus is: 0.90, 0.94 (our method); 0.93, 0.95 (MERLIN, true structure) and 0.86, 0.91 (MERLIN, discarded structure).

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Fine mapping of a region linked to low high-density lipoprotein cholesterol on chromosome 16q23-24. *J. Lee[1], E. Suviolahti[1,3], A. Jokiaho[1], A. Huertas-Vazquez[1], R.M. Cantor[1], E. Sobel[1], R. Mar[1], T.W.A. de Bruin[4], A.J. Lusis[1], M.-R. Taskinen[2], P. Pajukanta[1].* 1) Dept of Human Genetics, David Geffen School of Medicine at UCLA; 2) Dept of Medicine, Helsinki University Central Hospital, Finland; 3) Dept of Molecular Medicine, National Public Health Institute, Finland; 4) Dept of Internal Medicine, Maastricht University, Netherlands.

Low level of plasma high-density lipoprotein cholesterol (HDL-C) is a major risk factor for coronary heart disease and a component trait of familial combined hyperlipidemia (FCHL). Linkage of the low HDL-C trait to the 16q23-24 region has previously been observed in the combined analysis of Finnish and Dutch FCHL families and in Mexican American families. We used a two-step strategy to fine map a 12.4-Mb region with LOD scores within 1.0 of the top LOD scores in the combined Dutch/Finnish and Mexican American linkage analyses. First, a dense set of SNPs are tested for association in the Finnish and Dutch families. Second, SNPs with preliminary association signals are confirmed in an independent Caucasian case-control study sample. To ensure selection of the most informative and non-redundant SNPs, we selected tagSNPs utilizing the HapMap data and the LD Select program. A total of 1411 SNPs comprised of 1348 tagSNPs with MAF 0.1, representing a SNP density of 5 kb, and 63 nonsynonymous SNPs were genotyped in Finnish and Dutch families. These SNPs were analyzed for association given linkage using the FBAT program. In the first step, analysis of the Finnish families identified two genes, spaced ~10 Mb apart, with clusters of several associated, non-redundant SNPs per gene with P-values of 0.0005-0.01. When analyzing the Dutch and Finnish families together, only one of these two genes exhibited further support for association. Currently, we are testing the associated SNPs in an independent Caucasian low HDL-C case-control study sample to reduce false positive results and confirm the association. To conclude, in our preliminary analysis of 1411 SNPs for the 12.4-Mb region on 16q23-24, two genes show evidence for association with low HDL-C, and efforts to confirm the associated gene(s) are underway in case-control samples.

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Genome scan of age of onset of Alzheimers disease: Preliminary findings in Caribbean Hispanic families. *I. Chacon[1], H.S. Lee[1], V. Santana[1], J. Williamson[1], R. Lantigua[2], M. Medrano[2], E. Rogaeva[2], P. St George-Hyslop[3], R. Mayeux[1], J. Lee[1].* 1) Taub Inst & Sergievsky Ctr, New York, NY; 2) Univ Tecnologica de Santiago, Santiago, Dominican Republic; 3) Center for Research in Neurodegenerative diseases, Univ of Toronto, Toronto, Canada.

Alzheimer disease (AD) is the most common form of dementia and its risk increases with age. To date, three known AD genes, namely APP, PS1 and PS2, are found primarily in early onset of AD. In late onset AD, apolipoprotein E (APOE) has shown that genes can influence age at onset (AAO), and there likely to be other genes with similar function. Thus, AAO phenotype may enhance power to detect AD susceptibility genes by allowing us to look for genes that increase AD risk by lowering AAO. To localize genes related to AAO of AD in Caribbean Hispanic families, we conducted a genome scan on 1062 family members from 210 Caribbean Hispanic families with at least two affected individuals and recruited through AD probands. We genotyped 430 microsatellite markers across the genome with an average distance of 10 cM. To perform linkage analysis on AAO as a quantitative trait, we used the variance component approach as implemented in SOLAR. AAO was computed two ways. First, we used AAO if affected and age of last examination if unaffected, while adjusting for AD status, sex, education and APOE genotype. Second, we used the residual from a Cox regression model after adjusting for sex, education, and APOE. Our linkage analysis under the first definition revealed that 1q32-q41, 5q-ter, 6p11.2, 11q22, 12q24.1-.2, 17p12-17q11.1 and 21q21-22 were significant. When we used the second definition of AAO, the LOD scores decreased somewhat but the linkage remained significant for 5q-ter, 6p11.2 and 21q21-22. We did not observe linkage on 10q that was previously reported by us and others for both AD as well as onset phenotypes. However, we did observe linkage in regions (e.g., 12q24.1 and 17p12-17q11.1) previously reported by others using Caucasian samples. Our results show that AAO can be an important phenotype to search for AD susceptibility genes; however, further investigations are needed to confirm linkage to these locations.

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First evidence that one or more rare genetic polymorphisms with high penetrance may be involved in the etiology of endometriosis. *K.T. Zondervan[1], J. Lin[2], G. Dawson[3], D. Zabaneh[3], V. Smith[3], S.T. Bennett[3], A. Lambert[2], A. Carey[3], D.E. Weeks[4], S.A. Treloar[5], G.W. Montgomery[5], D.R. Nyholt[5], N.G. Martin[5], L.R. Cardon[1], I.J. Mackay[3], J. Mangion[3], S.H. Kennedy[2].* 1) Wellcome Trust Cntr Human Genet, Univ Oxford, UK; 2) Nuffield Dept Obstet Gynaecol, Univ Oxford, UK; 3) Oxagen Ltd, Abingdon, United Kingdom; 4) Dept Human Genet, Univ Pittsburgh, USA; 5) Queensland Institute of Medical Research, Brisbane, Australia.

Endometriosis (endometrial-like deposits outside the uterus) is a common complex disease associated with pelvic pain and subfertility in women. Its etiology is unknown, but familial aggregation has been shown in humans and non-human primates. For a genome-wide linkage study in Oxford (UK), 256 Caucasian families with 2+ sister-pairs with endometriosis were recruited; 52 families contained 3 or more affecteds. Non-parametric genome-wide linkage analyses in the 52 families were conducted, using sex-specific information from the Rutgers map. Parametric maximum LOD (MOD) scores were found by iterating across different inheritance models. Results were compared with those in a replication sample of 196 Caucasian families from Australia. Six non-parametric LOD score peaks with K&C LODs 1 were found. One of the peaks, on chromosome 7, reached a LOD of 3.49 (genome-wide p-value: 0.007). Parametric analyses showed MOD scores at 58.4 cM for 3 models: 1) allele frequency = 0.002 (MOD= 3.66); 2) 0.005 (MOD = 3.73), and 3) 0.07 (MOD = 3.91); penetrances 0.4-0.6; proportion of linked families : 0.87-0.97. In the Australian sample, the 3 models showed peak heterogeneity LODs (HLODs) at 46.0 cM of 1.02, 0.83 and 0.61 (: 0.17-0.23). The combined UK-Aus analysis showed 1 peak at 53.3 cM, with max HLODs of 2.51, 2.43, and 1.91. Further phenotypic analyses showed significant associations between the posterior probability of linkage for individual families and the severity of disease, likely to account (in part) for the different magnitude of results between the two samples. The results suggest that one or more rare genetic polymorphisms on chromosome 7 with moderate to high penetrance are involved in the aetiology of a subgroup of women with endometriosis.

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A gene-centric approach to fine-mapping a type 2 diabetes gene on chromosome 10q23-26. *T.M. Frayling[1], S. Heath[2], J.G. Garnier[2], M. Foglio[2], D. Lechner[2], C.J. Groves[3], S. Wiltshire[3], M.N. Weedon[1], G. Hitman[4], M. Walker[5], M.I. McCarthy[3], M. Lathrop[2], A.T. Hattersley[1], I.G. Gut[2].* 1) Peninsula Medical School, University of Exeter, United Kingdom; 2) Centre National de Genotypage, Evry, France; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK; 4) Queen Mary School of Medicine and Dentistry, University of London, UK; 5) School of Medicine, Newcastle upon-Tyne, UK.

There is strong evidence that a type 2 diabetes gene exists on chromosome 10q23-26. The region is one of the thirteen highest regions in a combined analysis of twelve type 2 diabetes genome scans. The region has the second highest LOD score (1.99) in the UK-based Warren2 genome scan of 573 affected sibships. The evidence for linkage is disproportionately provided by the subset of 245 sibships diagnosed 55 years (LOD = 3.07, p = 0.02 for increase vs. 573). The type 2 diabetes gene on 10q has not been found. We selected SNPs in genes from the 24MB region designated by the flanking microsatellites of the region of linkage (LOD >0.59). We genotyped a panel of 1536 non-redundant hapmap single nucleotide polymorphisms (SNPs) in 420 unrelated UK controls and 437 unrelated cases using the illumina bead-station. Cases were selected from sibships with the best evidence for linkage. We assessed how well successful SNPs captured common variation across the region by calculating pairwise r^2 values between genotyped SNPs and non-genotyped phase1 hapmap SNPs. We excluded SNPs with less than 80% calls and those with significant deviations from Hardy-Weinberg equilibrium in controls after Bonferroni correction. The resulting 1378 SNPs captured 57%; and 76%; of all Phase1 hapmap SNPs in the region at r^2 0.8 and r^2 0.5 respectively. Allelic tests identified 58 nominally significant SNPs (p 0.05). Thirty of these SNPs had higher odds ratios using the 206 cases from families diagnosed before age 55 compared to the 231 cases from the families diagnosed after 55. These thirty SNPs occurred in twelve genes, three of which, TLL2, KCMA1 and COX15 are expressed in the islets of langerhans. Further analyses are needed to more fully examine these and other genes in the region.

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Genetic studies of stuttering in a founder population. *J.K. Wittke-Thompson[1], N. Ambrose[2], E. Yairi[2], C. Roe[1], C. Ober[1], N.J. Cox[1, 3].* 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Speech and Hearing Science, University of Illinois Urbana-Champaign, Champaign, IL; 3) Department of Medicine, The University of Chicago, Chicago, IL.

We conducted genome-wide linkage and association analyses to identify genetic determinants of stuttering in a founder population, in which 48 individuals affected with stuttering are connected in a single 232-person genealogy. A novel approach was devised to account for all relationships within four smaller sub-pedigrees from this genealogy, because the size of the pedigree including all affected individuals would have been prohibitively large for multipoint linkage analyses to be conducted. Regions on chromosomes 1, 3, 5, 9, 13, and 15 had evidence of linkage to stuttering, with the three strongest linkage signals on chromosome 3 (P = 0.013, 208.8 cM), chromosome 13 (P = 0.012, 52.6 cM), and chromosome 15 (P = 0.02, 100 cM). The three regions with the strongest evidence for association with stuttering that overlapped with a linkage signal are located on chromosome 3 (P = 0.0047, 195 cM), chromosome 9 (P = 0.0067, 46.5 cM), and chromosome 13 (P = 0.0055, 52.6 cM). In order to locate regions showing evidence for linkage across studies, we conducted the first meta-analysis for stuttering and identified regions on chromosomes 2, 3, 5, 7, 9, 13, and 15 with evidence for linkage in both the Hutterites and The Illinois International Genetics of Stuttering Project. The two regions showing the strongest evidence for linkage in the meta-analysis were on chromosome 5 (P = 0.0051, 105-120 cM; P = 0.015, 120-135 cM) and chromosome 2 (P = 0.013, 180-195 cM). The current findings identify several new regions with evidence for susceptibility to stuttering, as well as replicate results from previous studies concerning stuttering or language disorders, including a region on chromosome 13 that was previously implicated in Specific Language Impairment.

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Incorporating length of peak in test statistics for disease gene mapping. *B.P.C. Koeleman[1], F. Dudbridge[2].* 1) Dept Medical Genetics, UMCU, Utrecht, Netherlands; 2) MRC Biostatistics Unit, Cambridge, UK.

Genome wide scans are frequently used to locate disease gene regions, either by family based linkage analysis, or, more recently, by case-control association. The result of such analyses is a number of regions containing peaks of increased statistics. Particularly in the context of complex genetic disorders, for which more than one susceptibility gene is expected, the question arises of which of the multiple peaks represent true signals and which are false. A promising argument was presented by Terwilliger et al (Am J Hum Genet 61:430-8), who used renewal theory and simulations to show that length of a true peak is expected to be longer than that of a false peak of similar magnitude. Subsequently, Visscher and Haley (Genet Epidemiol 20:409-14) argued that this increased length is not a property of disease risk and that there is no additional information in the length of a peak to distinguish loci with true effects from those with no effect. However their results do not contradict the original argument. Also, a simulation study exploring power to detect true disease regions with statistics that incorporate length of peak has not been performed. Here we perform such a simulation study. For linkage analysis we used the area under the multipoint linkage curve. We explored different scales of the length and height dimensions. For association analysis, a similar area was calculated using the distance between markers showing significant association and a function of the association p-value. A peak was defined as all markers showing significance at the nominal level around the most associated marker of a screen, and allowance was made for gaps of one non significant marker. Our results show that incorporating length of peak into the test statistic can result in significantly improved power to detect a true disease locus. Power of area statistics seemed to converge to that of height statistics at around 80%, which may be improved by using different scalings of the length. While most studies remain underpowered to detect the low risk susceptibility genes that are expected to underlie complex disorders, length of peak statistics may prove very valuable.

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Analysis of 5319 SNPs covering regions of linkage to autism on chromosomes 5 and 17. *J. Stone[1], B. Merriman[1], D.H. Geschwind[2,4], S. Nelson[1,3].* 1) Dept Human Genetics; 2) Program in Neurogenetics, Dept of Neurology; 3) Dept of Psychiatry and Biobehavioral Sciences; 4) Center for Autism Research, Neuropsychiatric Institute, University of California-Los Angeles, Los Angeles.

Autism is a complex disorder identified by impaired language and social skills, restricted repetitive behaviors, and an early childhood onset. Previously, our group has identified a region of linkage to chromosome 5 (MLS 2.54, Yonan et al. 2003) and a region of male-specific linkage to chromosome 17 (MLS 4.3, Stone et al. 2004) using multiplex families collected by the Autism Genetic Resource Exchange (AGRE). We have fine-mapped these two regions with high density SNPs - 3366 SNPs on chromosome 5 and 1953 SNPs on chromosomes 17 - in 227 trios from the AGRE resource. A total of 17.2 MB and 13.5 MB of non-repetitive sequence are analyzed on chromosomes 5 and 17, respectively. Cumulatively over both regions, 204 known or proposed genes along with the intergenic spaces were included in this study. SNPs on chromosome 5 were individually analyzed for association with transmission disequilibrium test (TDT) followed by haplotype block analysis using all 227 families. The analysis of chromosome 17 was restricted to the 140 trios with only male affected children, as these are primarily responsible for the linkage signal. SNP and haplotype analysis on chromosome 5 has provided evidence for association to 20 regions/haplotype blocks, 8 of which are located in known genes, at an uncorrected p-value < 0.01. Analysis of transmission bias on chromosome 17 has resulted in evidence for association at an uncorrected p-value < 0.01 for 11 regions/haplotypes. These regions include 5 known genes and 5 unconfirmed genes with no available functional information from public databases. Here we present the results from a first stage, high density screen for association under two regions of linkage to autism. The nominal associations detected here are being tested in a replication sample to eliminate expected false positives inherent in a high density SNP screen.

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Linkage scans for HDL-C, Triglycerides and Apolipoprotein AI in three populations and comparison with the NHLBI GeneLink meta-analyses. *K.L.E. Klos[1], S.L.R. Kardia[2], S.T. Turner[3], C. Hanis[1], L.A. Cupples[4], S.C. Hunt[5], M.C. Mahaney[6], S.R. Patel[7], M.A. Province[8], T. Rice[8], G.S. Berenson[9], E. Boerwinkle[1].* 1) Dept Human Genetics, Univ Texas, Houston, TX; 2) University of Michigan, Ann Arbor, MI; 3) Mayo Clinic, Rochester, MN; 4) Boston University, Boston, MA; 5) University of Utah, Salt Lake City, UT; 6) Southwest Foundation for Biomedical Research, San Antonio, TX; 7) Brigham and Women's Hospital, Boston, MA; 8) Washington University School of Medicine, Saint Louis, MO; 9) Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

We used linkage analysis to identify regions of the genome with evidence of quantitative trait loci that influence variation in plasma levels of Apolipoproteins AI (ApoAI), High-Density Lipoprotein-Cholesterol (HDL-C) and Triglycerides (TG) in sibships of the Genetic Epidemiology Network of Arteriopathy (GENOA) study. The strongest evidence of linkage was LOD = 3.68 on chromosome (chr) 18 for plasma ApoAI level in African-Americans. LOD scores greater than 2.0 were also observed on chr 3 for HDL-C (LOD = 2.12) and chr 4 for lnTG (LOD = 2.42) in European-Americans; and on chr 11 for HDL-C (LOD = 2.28) in Mexican-Americans. The chr 11 HDL-C peak in Mexican-Americans is supported by an overlapping HDL-C LOD peak of 1.31 at 21 cM on chr 11 in the NHLBI Family Heart Study. Statistically significant evidence of linkage for TG levels (LOD = 3.55 at 45 cM on chr 20) in Caucasians of the Framingham Heart Study overlaps with suggestive evidence (LOD = 1.93 at 42 cM) in GENOA African-Americans. An HDL-C peak on chr 16 (LOD = 4.35 at 102 cM) in the San Antonio Family Heart Study (SAFHS) was not replicated in GENOA. However, in a second GENOA analysis ApoAI and TG level was incorporated into the HDL-C adjustment to approximate the SAFHS adjustment, resulting in a LOD = 3.16 at 95 cM in African-Americans. We compare these results with the GeneLink meta-analysis (https://genelink.nhlbi.nih.gov/index.jsp).

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Genetic linkage analysis of osteoarthritis at multiple joint sites in Dutch sibpairs. *J.L. Min[1], I. Meulenbelt[1], N. Riyazi[2], M. Kloppenburg[2], J.J. Houwing-Duistermaat[3], A.B. Seymour[4], N. Lakenberg[1], C.M. van Duijn[5], P.E. Slagboom[1].* 1) Molecular Epidemiology, Leiden University Medical Centre, The Netherlands; 2) Rheumatology, LUMC, The Netherlands; 3) Medical Statistics, LUMC, The Netherlands; 4) Pfizer Global Research and Development, Groton, USA; 5) Epidemiology & Biostatistics, Erasmus Medical Centre, The Netherlands.

Osteoarthritis (OA) is a common late-onset joint disease and is an important cause of pain and disability in the general population. Previous studies have suggested a strong genetic component to multiple definitions of OA, which appears to be transmitted as a complex and multifactorial trait. Association studies and genome wide scans in OA-affected subjects have revealed multiple positive linkage areas and susceptibility loci. In our studies, consisting of OA-affected sibpairs (the GARP study) and a population-based cohort (the Rotterdam study), associations were found with variants within the *FRZB* and the *MATN3* gene with different OA phenotypes. In these studies, we focus on the localisation and identification of susceptibility loci for OA at multiple joint sites, which systemic phenotype thus far received little attention. We have performed a genomewide scan in 191 sibpairs from the GARP study and in 79 sibpairs from the Rotterdam study. The GARP study consists of Caucasian sibpairs of Dutch ancestry with predominantly symptomatic familial OA at multiple joint sites. The sibpairs from the Rotterdam study consist of Dutch probands and their siblings with radiographic OA at multiple sites (heritability 0.78, Bijkerk et al. 1999). Previously, we have shown association of the *FRZB* locus in the GARP study corresponding to an odds ratio of 1.6 (95% CI, 1.1-2.3). Furthermore, we observed associations of the *MATN3* locus corresponding to an odds ratio of 2.9 (95% CI, 1.2-7.3) for spinal OA in the Rotterdam study and 2.0 (95% CI, 1.3-3.1) for CMC1 OA in the GARP study. Since these loci do not explain the genetic component, a complete genome scan using the Human Linkage Set v2.5 MD10 (Applied Biosystems), containing 399 microsatellite markers with an average spacing of 10cM was recently finished. Results of the linkage analysis will be presented.

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A novel bayesian approach to localising disease genes. *J. Marchini, S. Myers, G. McVean, P. Donnelly.* Mathematical Genetics Group, Department of Statistics, Oxford University, Oxford, UK.

Many association studies will not actually type the disease locus. Instead, they will rely on linkage disequilibrium between the true but untyped disease locus and the markers which are typed in the study. There has been considerable discussion about whether to test each SNP separately, or to use haplotype information, and if so, in which ways. In effect, haplotype-based tests aim to capture the variation at SNPs not typed in the study. Here we describe a different approach to the problem. We use local haplotype information to impute genotypes at loci not explicitly typed in the study, and then base inference on these inferred genotypes. The imputation relies on a new "hidden SNP simulator", which uses a computationally convenient approximation to the coalescent. Conditional on the imputed genotypes, we fit a disease model using Bayesian logistic regression. With dense SNP data around a mapped locus, our method can be used for fine mapping and seems to outperform existing coalescent-based approaches. Perhaps more importantly, because it avoids reconstructing coalescent trees, it is very much faster (by about two orders of magnitude compared to the best coalescent method), and so applicable to much larger data sets. But the next generation of genome-wide association studies, for example those based on the HapMap, will give dense genotype information across much or all of the genome. In this setting, which motivated our approach, localisation is essentially the same as traditional fine-mapping, and our new approach provides a novel and efficient route to the detection of disease susceptibility loci.

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Fine-mapping reveals two SNPs associated with multiple sclerosis in an Australian sample of 359 case-relative trios. *J. Stankovich[1], R.B. Tan[1], M. Bahlo[1], J.P. Rubio[2], S.J. Huxtable[1], C. Cox[3], H. Butzkueven[2], I. van der Mei[4], T.J. Kilpatrick[2], T.P. Speed[1], S.J. Foote[1,4].* 1) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 2) Howard Florey Institute, University of Melbourne, Victoria, Australia; 3) GlaxoSmithKline, Harlow, United Kingdom; 4) Menzies Research Institute, University of Tasmania, Australia.

In a previous genome-wide scan of Tasmanian multiple sclerosis (MS) cases and controls, we identified haplotypes associated with multiple sclerosis on chromosomes 6q and 10q. After genotyping with additional microsatellite markers, the associations were replicated and the regions of association were narrowed. The two regions, of lengths 3.7 Mb and 1.3 Mb, have now been fine-mapped with 630 SNPs and 222 SNPs respectively. 63 case-parent trios from the original Tasmanian sample were genotyped, plus an additional 198 case-parent trios and 98 case-parent-sibling trios from the neighbouring Australian state of Victoria.

The SNP with the strongest association signal was at the distal end of the 1.3Mb region (nominal p=0.0004, corrected p=0.06 after permutation testing). This SNP tags a five-SNP haplotype that was slightly more strongly associated (p=0.0003). A SNP at the proximal end of the 1.3Mb region also displayed a strong association (p=0.0005). These two SNPs are currently being genotyped in a further 681 Victorian MS cases and 738 unrelated controls.

480 of the 852 SNPs we genotyped in the two regions were also genotyped in Phase I of the International HapMap project. For these 480 SNPs patterns of linkage disequilibrium in the 30 Caucasian HapMap trios were compared with patterns of LD in 30 Tasmanian and 30 Victorian case-parent trios. LD patterns are very similar in the Tasmanian and Victorian samples; LD patterns in the HapMap trios differ much more markedly.

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Resequencing & fine map association study of G72/G30 region in bipolar disorder. *M. Maheshwari[1], L. Chunyu[2], J.A. Badner[2], C.K. Smith[1], S.L. Chrisitian[2], H. Zou[2], S.D. Detera-Wadleigh[3], T. Bonner[4], F.J. McMahon[3], E.S. Gershon[2], R.A. Gibbs[1].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston,TX; 2) Department of Psychiatry, University of Chicago, Chicago IL; 3) MAP Genetics, Mood & Anxiety Disorders Program, National Institutes of Health,Bethesda,MD; 4) Laboratory of Genetics, National Institutes of Mental Health, Bethesda, MD.

Earlier we reported association between G72/G30 gene locus on 13q33 and bipolar illness (Hattori et al., Am J Hum Genet 72, 1131-1140, 2003). In this study, we resequenced the entire 50 kb region of G72/30 genes in 11 affected individuals of bipolar families. 50 kb of genomic region was resequenced using big dye terminator sequencing chemistry and analyzed using Sequencher software. We discovered 106 SNPs in this region. Of these, SNPs in and around exons, within conservative genomic regions or previous association signals were selected for further genotying. Genotyping was done in two large pedigree sets- the Intramural NIMH Clinical Neurogenetics Branch (CNG) & the NIMH Genetics Initiative (NIMH) pedigrees. 34 SNPs in CNG series and 27 SNPs in NIMH series were genotyped. Genotyping was done using SNPlex and/or TaqMan assays. Genotype data was tested for significance of association using ASPEX/Sib_tdt and EATDT analyses. Several SNPs were significant in CNG data set by TDT as compared to 1 SNP in NIMH dataset which did not overlap with CNG data set suggesting different variant alleles may account for two population sets. The EATDT analysis in the CNG series had one SNP that was significant by permutation, and several SNPs and haplotypes that were nominally significant. In the NIMH series, nothing was significant by permutation although several haplotypes were nominally significant. Conclusions: Significant association signals with additional SNPs confirm the presence of a susceptibility variant for bipolar disorder in the vicinity of the G72/G30 genes.

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Trivariate linkage analysis in SOLAR. *D. Warren[1], J.M. Soria[2], A. Buil[2], J.C. Souto[2], J. Fontcuberta[2], J. Blangero[1], L. Almasy[1].* 1) Genetics, SFBR, San Antonio, TX; 2) Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Multivariate linkage analyses allow simultaneous examination of linkage for multiple traits. Whereas this approach is often used to investigate pleiotropy, multivariate linkage screens may also improve power relative to univariate screens. Recent extensions of SOLAR's maximum-likelihood, variance components based linkage analysis routines permit three or more quantitative traits in models. Here, we use data from 396 individuals in 21 Spanish families enrolled in the GAIT study to illustrate the potential of trivariate linkage screens. We used genome-wide uni-, bi-, and trivariate linkage screens using 485 microsatellite markers spaced at 7.1 cM to localize quantitative trait loci (QTLs) influencing variation in clotting factor X (FX), a vitamin K-dependent protein involved in blood coagulation. Additional measured covariates included age, sex, and exogenous hormone use. All LODs were corrected to a standard equivalent to 1 degree of freedom. In the univariate screen, a maximum multipoint LOD of 1.25 occurred on chromosome 2q. A bivariate linkage screen was then conducted using FX and another vitamin K-dependent protein, clotting factor VII (FVII). FVII had marginal genetic (0.31, $p = 0.06$) but significant environmental (0.69, $p < 0.01$) correlations with FX. This yielded a maximum multipoint LOD of 4.14 near marker D13S285 on chromosome 13q, in the region of the FX structural gene. A third hemostasis-related phenotype, functional protein S (PS), was then added to the linkage model. PS is also vitamin K-dependent, and had significant genetic (0.59, $p < 0.01$) and marginal environmental (0.25, $p = 0.07$) correlations with FX. Using the trivariate approach, we estimated a maximum multipoint LOD of 4.71 on chromosome 13q, again near marker D13S285. QTL effect size estimates provide marginal evidence for a FX QTL and strong evidence for a FVII QTL at this locus. QTL-specific genetic correlations are not different from zero, suggesting that the amplified linkage signal of the trivariate screen is not due to pleiotropic effects of a chromosome 13q QTL. Instead, residual genetic and/or environmental correlations may underlie the linkage signal.

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Familial aggregation, linkage, and association analyses of autoantibody traits in systemic lupus erythematosus (SLE). *P.S. Ramos[1], J.A. Kelly[2], C. Gray-McGuire[3], R. Graham[4], C. Gillett[1], K. Espe[1], W. Ortmann[1], G. Bruner[2], T.W. Behrens[1], J.B. Harley[2], K.L. Moser[1].* 1) University of Minnesota, Minneapolis, MN; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Case Western Reserve University, Cleveland, OH; 4) Broad Institute, Boston, MA.

The goal of this study was to identify genes that contribute to SLE using autoantibody traits as intermediate phenotypes. We performed familial aggregation and linkage studies using our Oklahoma collection consisting of 229 multiplex SLE pedigrees. We found that a large percentage of unaffected individuals in these families have significant titers of a variety of autoantibodies. There is also strong familial aggregation of antinuclear antibodies (ANAs), anti-dsDNA, anti-La, anti-Ro, anti-nRNP, IgM aPL antibodies (Abs) and low titer rheumatoid factor. Genome-wide linkage analysis was performed for the following autoantibodies: Ro, La, nRNP, Sm, dsDNA, rheumatoid factor, ANAs and antiphospholipid Abs. Using an approach to rule out likely false positives and adjusting for multiple comparisons, we found evidence for linkage to anti-La Abs on 3q21 (adjusted P-value=1.9x10-6), to anti-nRNP and/or anti-Sm Abs on 3q27 (P=3.5x10-6), to anti-Ro and/or anti-La Abs on 4q34-q35 (P=3.4x10-4), and to anti-IgM aPL Abs on 13q14 (P=2.3x10-4). Another 45 loci of interest were also identified. Comparison of these results with loci identified in several autoimmune diseases revealed that 4q35 has also been previously implicated in autoimmune skin disorders and subsets of SLE patients with dermatological manifestations. The interferon regulatory factor 2 (IRF2) gene was chosen for initial association studies in our Minnesota collection of 215 SLE pedigrees. Four single nucleotide polymorphisms (SNPs) were evaluated using the Pedigree Disequilibrium Test (PDT). Association of a coding variant (rs3775543) was found using the following pedigree subsets: malar rash (P-value=.027), discoid rash (P=.043), oral ulcers (P=.005), photosensitivity (P=.016) and anti-Ro and/or anti-La (P=.005). Our studies show that autoantibody production in SLE is a complex trait and suggest IRF2 may play a role in dysregulation of IFN-related pathways in SLE.

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A QTL analysis of macrocephaly in autism families. *S. Spence[1], N. Kono[2], D.H. Geschwind[1], R.M. Cantor[2].* 1) UCLA Depts of Neurology & Psychiatry, Program in Neurogenetics, Center for Autism Research & Treatment; 2) UCLA Dept of Human Genetics, LA, CA.

Autism Spectrum Disorder (ASD) is a highly heritable complex neuropsychiatric syndrome, yet results of susceptibility gene searches have been modest and inconsistent due to its genetic and phenotypic heterogeneity. Macrocephaly (occipital frontal head circumference (OFC)98%) is a common feature in ASD that is highly heritable in the general population. In a sample of 200 nuclear families ascertained for at least two children with ASD from the Autism Genetic Resource Exchange, a significantly increased incidence in macrocephaly (20%) was observed, occurring equally in ASD probands and their non-autistic 1st degree relatives. OFC was also correlated in siblings ($r=.26$, $p<.0001$) and with their mid-parent values ($r=.30$, $p<.0001$) supporting its familiality in the ASD population. We hypothesized that analyzing age and sex adjusted head circumference as a quantitative trait within ASD pedigrees could identify loci harboring genes contributing to ASD risk through this phenotype. A quantitative trait locus (QTL) analysis was performed on OFC percentile measurements in 233 sibpairs from 134 pedigrees that included both ASD and non-ASD sibs. Because the distribution was skewed and the median OFC at the 80th %ile, the QTL analysis was conducted using the nonparametric option of the Genehunter software. Scores with a p-value of <.05 were found at 7 loci on 5 chromosomes: chr 1 @ 1p36($p=.04$) and 1p31.3($p=.04$); chr 2 @ 2p25.1($p=.02$) and 2q22($p=.005$); chr 8 @ 8q13.2-8q21.3($p=.03$); chr 10 @10q25.2($p=.04$); chr 18 @18p11.32($p=.02$). The 2q22 QTL is near a locus related to language delay in ASD. The 10q25.2 QTL is near PTEN, a gene implicated in various overgrowth syndromes and found to have mutations in macrocephalic patients with ASD. Those with macrocephaly and normocephaly did not differ on measures of cognition, language and behavior. We conclude that these loci may contain genes predisposing to macrocephaly in ASD. Given the frequency of the occurrence of this trait in ASD families (increased 10-fold over the general population) we propose that these genes may also confer risk to ASD.

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MCMC linkage analysis with multiallelic trait loci. *E.A. Rosenthal, E.M. Wijsman.* University of Washington, Seattle, WA.

Complex traits are influenced by multiple contributing genes. One approach to detect linkage is to allow for multiple underlying loci. Another is to focus on quantitative phenotypes. A method that incorporates both approaches is the reversible jump Markov chain Monte Carlo (RJMCMC) joint linkage and segregation analysis that is implemented in the package Loki: the number of quantitative trait loci (QTLs) need not be specified so that complex traits with unknown etiology can be modeled more adequately than with other methods. However, virtually all known trait genes have more than two alleles with respect to phenotypes of interest. One possible additional hindrance to linkage detection may be that current methods are either restricted to, or are carried out under, the assumption of underlying diallelic genes.

We propose an algorithm for extending the RJMCMC process to allow for an unspecified number of alleles at the underlying QTLs under the assumption of additivity for QTLs with three or more alleles. The algorithm includes three steps. The first is a transition between dominant and additive diallelic QTLs. This involves equating the genetic variances of the current and proposed QTL states and then calculating the additive and dominance variances of the proposed state while keeping the allele frequencies the same. The second step is the birth or death of an additive diallelic QTL which increases the acceptance rate of additive QTLs. This involves generating or removing, respectively, the additive genetic variance and allele frequencies at the QTL. The third step is the birth or death of an allele at additive QTLs. The frequency of a new allele is generated via sampling from the Dirichlet(1) distribution and subsequent normalization of all allele frequencies. An effect for the new allele is also sampled. The death of an allele involves its removal as well as the adjustment of the remaining allele frequencies. For this last step, the genetic variance of the current and proposed QTLs are not necessarily equal. Upon implementation, the algorithm may help in detecting multiallelic trait loci. [Supported by NIH HG 00035 and GM 46255.].

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Searching for genetic variations associated with schizophrenia in the *GRID1* gene region on chromosome 10q23.
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Schizophrenia (SZ, [MIM 181500]), with a prevalence ~1% worldwide, is a complex psychiatric disorder with many lines of evidence supporting a strong genetic component. To reduce genetic heterogeneity, we performed a genomewide linkage scan on 29 multiplex families of Ashkenazi Jewish (AJ) descent (Fallin *et al*. Am. J. Hum. Genet. 73:601-611, 2003), and got the strongest linkage signal at chromosome 10q22 (NPL score: 4.27, empirical P = 0.00002). *GRID1* (glutamate receptor, ionotropic, delta 1) is a candidate gene in this region, based on its predicted function as a glutamate receptor. In a subsequent family-based association study with AJ trios, we found independent evidence implicating the *GRID1* region ($P < 0.01$ for two SNPs and their haplotypes). Given these results, we initiated a search for a genetic variant(s) responsible for the association signal in and around the *GRID1* gene, which has 16 exons spanning 767 kb. The peak of our association study is in intron 2, which is about 157 kb in length and contains a microRNA (*hsa-miR-346*) whose mouse counterpart we find expressed in brain. We thus picked the first 4 exons of *GRID1*, the *hsa-miR-346* and additional 6 conserved non-coding regions in intron 2 of *GRID1* as our candidate segments and sequenced them in 10 AJ SZ probands and 3 AJ controls. We found 24 variants including 11 novel and 13 known SNPs. None of these is obviously pathologic and we are proceeding with an association study with ~600 AJ SZ patients and controls.

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Fine mapping a chronic obstructive pulmonary disease susceptibility locus on chromosome 2q. *C.P. Hersh[1,2], R. Lazarus[1], B.J. Klanderman[1], B.A. Raby[1], A.A. Litonjua[1], D.L. DeMeo[1,2], J.J. Reilly[2], D. Sparrow[3], S.T. Weiss[1], E.K. Silverman[1,2].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, MA; 3) Veterans Affairs Medical Center, Boston, MA.

Rationale: In the Boston Early-Onset Chronic Obstructive Pulmonary Disease (COPD) Study families, significant evidence for linkage of COPD-related traits has been demonstrated on chromosome 2q. We hypothesized that a high-resolution single nucleotide polymorphism (SNP) mapping strategy could be used to identify regions where COPD susceptibility genes are located.

Methods: With SNP genotype data from the HapMap project, we used a linkage disequilibrium (LD) tagging algorithm to select a set of 1536 SNPs across the 20 Mb linked region (1.5 LOD-unit support interval). These SNPs were genotyped on the Illumina platform in a case-control study comprised of cases with severe COPD from the National Emphysema Treatment Trial (NETT) and control smokers without airflow obstruction.

Results: 1402 SNPs were successfully genotyped (91.3%). These SNPs adequately captured the LD relationships across the region (mean of best LD with non-genotyped SNPs: $r^2 = 0.83$). Comparing 298 NETT cases to 317 control subjects, 103 SNPs were associated at $p<0.05$ and 23 at $p<0.01$ levels (smallest p-value = $2x10^{-5}$). 7 regions were identified with haplotype associations at $p<0.001$, including 2 regions with haplotype associations at $p<10^{-7}$.

Conclusions: Using a high-density SNP-based fine mapping approach, with both single SNP and haplotype analyses, we were able to identify several promising regions on chromosome 2q. Further genotyping will be necessary to confirm these associations and locate the functional variants that influence COPD susceptibility.

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Secondary Analyses of a Genome-wide Screen for Stuttering Susceptibility Loci. *R. Suresh[1,2], C.A. Roe[2], T.C. Gilliam[2], N.J. Cox[2,3].* 1) Dept. of Genetics & Development, Columbia Univ., New York, NY; 2) Dept. of Human Genetics, Univ. of Chicago, Chicago, IL; 3) Dept. of Medicine, Univ. of Chicago, Chicago, IL.

A genome-wide scan to identify loci that contribute to stuttering was carried out in 105 families, with at least two affected relative pairs. Genotyping was carried out with the Affymetrix 10k SNP chip. Primary analysis of the dataset showed highest evidence of linkage at chr. 9 at 60cM with a LOD score of 2.3. In order to assess potential interactions between this linkage signal and the rest of the genome, conditional analyses using weighting functions, generated based on the evidence for linkage in each family at this peak, was carried out. A strong positive interaction between the chr. 9 linkage signal and one on chr. 2 at 192.5cM ($p = 0.01$) was detected. This region on chr. 2 has been previously implicated in several linkage and association studies focusing on autism, and more specifically autism with phrase speech delay (*Shao Y, 2002*). There is also a significant sex bias in the incidence of stuttering, with a male:female ratio of approximately 2:1 during childhood, and increasing up to 4:1 or 5:1 in adulthood. Sex-specific analyses of the dataset indicated significant evidence for linkage with the female families (n=9) on chr. 21 (33.5cM) with a LOD score of 4.5 (genome-wide $p=0.017$). Analysis of the male families (n=74) showed suggestive evidence for linkage on chr. 7 (153.2 cM) with a LOD score of 2.99 (genome-wide $p=0.074$). These sex-specific findings were replicated in an independent stuttering dataset as well. Conditional analyses based on the chr. 7 linkage signal showed a positive interaction with chr. 12 at 114.5cM ($p = 0.001$). The location of the chr. 12 signal incidentally overlaps with the primary finding of whole-genome scan for stuttering in 46 highly consanguineous Pakistani families (*Riaz N, 2005*). Interestingly, a negative interaction between the chr. 7 linkage signal and the one on chr. 2 at 192.5cM was also detected ($p = 0.013$). The pattern of results is consistent with the possibility that there are two largely non-overlapping sets of families with different sets of genetic risk factors.

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Polymorphisms in the Vitamin K Epoxide Reductase (VKOR) gene are strongly associated with warfarin dosage requirements in patients receiving anticoagulation. *L.A. Lange[1], T. Li[2], X. Li[3], L. Susswein[1], B. Bryant[1], R. Malone[1], E.M. Lange[1], T.-Y. Huang[1], D.W. Stafford[1], J.P. Evans[1].* 1) University of North Carolina, Chapel Hill, NC; 2) Salk Institute for Biological Sciences, La Jolla, CA; 3) Scripps Research Institute, La Jolla, CA.

Warfarin is a mainstay of therapy for conditions which result in an increased risk of thromboembolic events. Yet the use of this common agent is fraught with complications, including major bleeding episodes and death. Little is known regarding inter-individual variation in warfarin response. We tested for association between single nucleotide polymorphisms (SNPs) in the recently identified VKOR gene (which encodes the target for warfarin activity) and weekly warfarin dose required to maintain patients at their desired anticoagulation target. The sample consisted of 115 (Caucasian, N=96; African American, N=19) patients from anticoagulation clinics at the University of North Carolina at Chapel Hill who were being treated with warfarin. A combination of direct sequencing and genotyping were performed, resulting in the identification of six SNPs in VKOR. Mean weekly warfarin dose required to achieve each patients target International Normalized Ratio (INR) was collected over a mean treatment period of 20.6 months. Analysis of covariance models and haplotype analysis (using Haplo.Stat) were performed. Three of the VKOR SNPs were found to be very strongly associated with the average warfarin dose required to achieve the target INR (p-values ranging from 0.0000021 to 0.0000095), with results similar for both race-stratified and combined samples. The range in mean weekly dose by genotype ranged from approximately 27mg (more common allele homozygote) to 47mg (less common allele homozygote), which is of considerable clinical interest. These three SNPs are in very strong linkage disequilibrium (LD) and haplotype analyses were consistent with single SNP results. The current results combined with the recent results of Rieder et al. (N Engl J Med) represent a potentially clinically useful proof-of-principal for the use of pharmacogenomic information in medicine and may lead to an improved understanding of warfarins actions.

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Common polymorphisms in the promoter of the Visfatin gene (PBEF1) in a french canadian population. *S.D. Bailey[1], K. Desbiens[2], P. Lepage[3], J. Faith[3], C. Doré[3], J. Fontaine[3], T.J. Hudson[1,3], C. Bouchard[4], L. Pérusse[5], D. Gaudet[6], M.C. Vohl[5], J.C. Engert[1,2].* 1) Human Genetics, McGill Univ., Montreal, QC; 2) McGill Univ. Health Centre, Montreal, QC; 3) McGill Univ. & Genome Quebec Innovation Centre, Montreal, QC; 4) Pennington Biomedical Research Centre, Baton Rouge, LA; 5) Laval Univ., Ste. Foy, QC; 6) Université de Montréal, Complexe hospitalier de la Sagamie, Chicoutimi, QC.

The occurrence of obesity and non-insulin-dependent diabetes mellitus (NIDDM) are highly correlated. The newly characterized adipokine visfatin has been shown to exhibit insulin mimetic effects and to correlate strongly with visceral adiposity. Therefore, genetic variants of visfatin are potentially involved in either obesity and/or NIDDM. We sequenced approximately 1000 bps of the promoter and all coding regions of the PBEF1 gene in 18 individuals with varying degrees of visceral fat from the Quebec Family Study (QFS) as well as five individuals with high waist to hip ratios selected from the Saguenay-Lac-Saint-Jean (SLSJ) region of Quebec. We identified one silent mutation in Exon 7 (SER301SER), but no missense mutations. This is in accordance with dbSNP, which lists the SER301SER variant but no other coding SNPs. However, our sequencing identified six intronic SNPs and four SNPs within the promoter region with allele frequencies from 0.02 to 0.50. Further sequencing of an 856 bp region of the promoter led to the discovery of five additional SNPs in the QFS population. To investigate whether visfatin gene variants play a role in obesity-related phenotypes or the pathogenesis of NIDDM, we have genotyped and analyzed 10 potential regulatory SNPs from the promoter region of the gene (the nine discovered through our sequencing and one additional SNP chosen from a database search). Using QTDT, an association was found between one of the genotyped SNPs (rs7789066) and triglyceride levels (p=0.0251) in the QFS study (approximately 930 participants). In addition, we genotyped this promoter SNP in an additional 360 individuals from an NIDDM case-control cohort from the SLSJ region of Quebec, but no association was found between NIDDM and this SNP.

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Haplotype Sliding Windows Approach in Chromosome 12q Yields Priority Association Regions for Asthma and Total IgE Levels in a Family-Based Population of African Descent. *A.V. Grant[1, 2], S. Zhang[1], P. Chi[2], D. Baltadzhieva[1], T. Berg[1], L. Shao[1], A. Zambelli-Weiner[1], E. Ehrlich[1], M.L. Stockton[1], P.N. Levett[3], R.G. Ingersoll[4], A.F. Scott[4], D.M. Fallin[2], R.A. Mathias[5], T.H. Beaty[2], J.G.N. Garcia[6], K.C. Barnes[1, 2].* 1) Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; 3) Faculty of Medicine, University of the West Indies, School of Clinical Medicine and Research, Barbados; 4) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 5) Inherited Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland; 6) Department of Medicine, University of Chicago Pritzker School of Medicine, Chicago, IL.

Previously, we reported evidence of linkage on chromosome 12q to asthma and high total IgE levels in African Caribbean families selected for asthma from Barbados. To fine map this region, we genotyped 750 SNPs spanning ~50 Mb in this 12q region in 670 individuals from 119 Barbadian families. The joint hypothesis of linkage and association between markers and two phenotypes (asthma, log[total IgE]) was tested using the Family Based Association Test. Two-six SNP sliding window haplotypes were examined. In the final set of 726 SNPs, regions of interest were defined by segments of contiguous SNPs within 500 kb of any SNP with a global p-value ≤ 0.05 for at least one window including that SNP or single-SNP association test. This strategy yielded significant regions for asthma comprising 354 SNPs, and 324 SNPs for log[total IgE]. Significant regions overlapped considerably for these two phenotypes. By graphically juxtaposing genes available from public databases with p-values from windowing for both phenotypes, we identified 14 genes, one of which is a candidate for other immune disorders (MYF5) , containing variants showing associations with p-values ≤ 0.05. Additional SNP fine-mapping is underway to adequately cover candidate genes identified in this study for asthma and atopy.

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Genetic Variation in ACDC and ADIPOR2 genes are Associated with Insulin Resistance Syndrome (IRS)-Phenotypes in Mexican Americans. *D.K. Richardson, J. Schneider, L.M. Rodriguez, R. Arya, M.P. Stern, R.A. DeFronzo, J. Blangero, R. Duggirala, C.P. Jenkinson.* San Antonio, TX.

Adiponectin (ACDC) is an adipocyte-derived hormone implicated in energy homeostasis, and insulin resistance. Two receptors for adiponectin have been identified (ADIPOR1 and -2) both of which are highly expressed in skeletal muscle. Analysis of genetic variants in the ACDC, ADIPOR1 and ADIPOR2 genes has not previously been reported in Mexican Americans (MA). We genotyped variants in the ACDC (n=3), ADIPOR1 (n=5), and ADIPOR2 (n=12) genes in the San Antonio Family Diabetes Study (SAFDS) population. We performed SNP-phenotype association analyses using the quantitative trait linkage disequilibrium (QTLD) test, within a variance components analytical framework as implemented in SOLAR. When a variant exhibited evidence of potential population stratification, we used the quantitative transmission disequilibrium test (QTDT). Linkage disequilibrium (LD) between SNP pairs was estimated using the absolute correlation coefficient |p|. Of 20 SNPs examined, 10 (ACDC=3, ADIPOR1=1, and ADIPOR2=6) were polymorphic. Several of these SNPs exhibited significant association with IRS-related traits. SNPs 29661, 33371, 33447, 33630 and 33775 in the ADIPOR2 gene were associated with triglyceride levels (P=0.009, 0.00024, 0.044, 0.047, and 0.0045). SNP 349 in ACDC was associated with sum of skin fold thickness (P=0.013). In addition, SNPs 29661 and 33630 of ADIPOR2, and SNP 276 of ACDC, were significantly associated with fasting plasma glucose concentrations (P=0.048, 0.044 and 0.015). SNP 712 of the ACDC gene showed evidence of potential population stratification and yielded a significant QTDT association with fasting plasma glucose concentration (P=0.002). For the ADIPOR2 variants, the minor alleles were associated with decreased triglyceride levels and increased fasting plasma glucose concentration. The minor alleles in the ACDC variants were associated with increased fasting plasma glucose concentration and an increase in sum of skin fold thickness. In conclusion, we have found evidence for association of variants in ACDC and ADIPOR2 genes with some components of the IRS in the MA population.

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Genetic analysis of polymorphisms in the growth factor receptor binding protein-2 and obesity phenotypes: the IRAS Family Study. *B.S. Sutton[1,2], C.D. Langefeld[3], J.K. Campbell[3], S.M. Haffner[5], J.M. Norris[6], L.E. Wagenknecht[3], D.W. Bowden[1,2,4].* 1) Ctr Human Genomics,Wake Forest Univ Sch Med, Winston Salem, NC; 2) Dept Biochem, Wake Forest Univ Sch Med, Winston Salem, NC; 3) Dept Public Health Science, Wake Forest Univ Sch Med, Winston Salem, NC; 4) Dept Internal Med, Wake Forest Univ Sch Med, Winston Salem, NC; 5) Dept Med, Univ of TX Health Science Ctr, San Antonio,TX; 6) Dept Preventative Med and Biometrics, Univ CO Health Science Ctr, Denver, CO.

Growth factor receptor binding protein 2 (GRB2) has been suggested to inhibit fat cell differentiation and to be involved in the leptin signaling pathway. Expression of GRB2 protein in obese insulin-resistant diabetic KKAy mice was dramatically decreased compared to non-diabetic mice. In a genome-wide scan of Hispanic families in the IRAS Family Study (IRASFS) significant evidence of linkage (LODs 2.5-3.1) was observed to several obesity phenotypes on chromosome 17q24-25, the location of GRB2. Thus, due to the suggested role of GRB2 in obesity and the genes location under the linkage peak, 9 polymorphisms were genotyped across the 87.5 kb GRB2 genomic region. SNPs were genotyped on 1425 individuals from 91 Hispanic families in the IRASFS. Linkage disequilibrium (LD) was high for all SNPs, D>0.97 suggesting GRB2 lies in a single LD haplotype block. SNPs were tested for association with six obesity phenotypes [BMI, waist, waist: hip ratio (WHR), subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and visceral: subcutaneous ratio (VSR; a measure of fat compartmentation/distribution)] by computing a series of generalized estimating equation (GEE1). Significant evidence of association to VSR was detected with the majority of the SNPs, with the remaining SNPs showing a trend of association (P-value 0.03-0.09). Quantitative pedigree disequilibrium test (QPDT) analysis complemented these results with eight SNPs showing single SNP association (or trending toward association; P-value 0.01-0.08). No additional obesity phenotype revealed evidence of association suggesting GRB2 may be involved in controlling the pattern of fat deposition in the Hispanic population.

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Genetic Predictors of Asthma Exacerbations among Children in the Childhood Asthma Management Program (CAMP) Study. *J.C. Celedon[1,2,3], K. van Steen[4], C. Lange[4], B. Raby[1,2,3], K. Tantisira[1,3], E.K. Silverman[1,3], S.T. Weiss[1,3].* 1) Channing Lab, Brigham & Women's Hosp; 2) Div. of Pulmonary/Critical Care Medicine, Beth Israel Deaconess Med. Ctr; 3) Dept. of Medicine, Harvard Medical School; 4) Dept. of Biostatistics, Harvard School of Public Health, Boston, MA.

Little is known about genetic determinants of asthma exacerbations. We examined the relation between 235 single nucleotide polymorphisms (SNPs) in 51 candidate genes and asthma exacerbation phenotypes among 1,990 members of 645 families of children with asthma in the CAMP Genetics Ancillary Study. Information on asthma exacerbation phenotypes (use of oral corticosteroids, visits to urgent care or the emergency department, and hospitalizations) was obtained initially at the two-month follow-up visit and then every four months for a total follow-up period of 60 months. The analysis of association between SNPs in candidate genes and asthma exacerbation phenotypes was performed with an extension of the Family-Based Association Test (FBAT) statistic for analysis of repeated measures (FBAT-PC), as implemented in the PBAT program (http://www.biostat.harvard.edu/clange/default.htm). Because corticosteroid treatment influences the development of asthma exacerbations, the analysis was stratified by treatment arm (inhaled budesonide vs. nedocromil/placebo). Single nucleotide polymorphisms (SNPs) in the gene for the corticotropin-releasing hormone receptor 1 [CRHR1]) were associated with asthma exacerbation phenotypes in both treatment groups ($P <= 0.03$ in all cases). Among families of asthmatic children treated with placebo or nedocromil, variants in the gene for T-box 21 (TBX21) were associated with asthma exacerbation phenotypes ($P <= 0.02$ in all cases). Among families of asthmatic children treated with oral corticosteroids, SNPs in the promoter of the gene for interleukin 10 (IL10) were associated with asthma exacerbation phenotypes ($P <= 0.0009$ in all cases). Similar results were obtained when the analysis was restricted to white individuals. Our results suggest that SNPs in three genes (CRHR1, TBX21, and IL10) interact with treatment in influencing the development of asthma exacerbations among children with asthma.

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Linkage disequilibrium mapping for Tourette Syndrome in two population isolates. *D. Keen-Kim[1], C.A. Mathews[2], S.K. Service[1], V.I. Reus[3], T.L. Lowe[3], L.D. Herrera[4], C. Budman[5], R.D. Bruun[5], E. Erenberg[6], A. Naarden[7], N.B. Freimer[1].* 1) Center for Neurobehavioral Genetics, University of California, Los Angeles, CA; 2) Department of Psychiatry, University of California, San Diego, CA; 3) Department of Psychiatry, University of California, San Francisco, CA; 4) Hospital Nacional de Ninos, San Jose, Costa Rica; 5) Movement Disorders Center in Psychiatry, North Shore University Hospital, Manhasset, NY; 6) Department of Neurology, Cleveland Clinic Foundation, OH; 7) Department of Neurology, University of Texas Southwestern Medical School, Dallas, TX.

Tourette Syndrome (TS) is a childhood disease characterized by uncontrollable movements and sounds (motor and vocal tics) that affects approximately one in 2000 individuals worldwide. The considerable genetic and clinical complexity of TS has made genetic mapping difficult in the past, and to date there are no known candidate genes with a clear role in the etiology of the disease. The Tourette Syndrome Association International Consortium on Genetics (TSAICG) recently completed genome-wide linkage scans of 252 unrelated sib-pair families (465 sib-pairs) and 23 large nuclear families. Separate and combined data set analyses identified several regions with genome-wide significant or suggestive linkage to TS. Recent studies have shown that linkage disequilibrium (LD) is detectable over much longer genomic segments within isolated populations than in older and more heterogeneous populations. We therefore hypothesize that mapping genes for complex traits such as TS is simpler in isolated than in outbred populations. To this end, we are fine-mapping four of the broad linkage regions identified in the TSAICG scans (chromosomes 2p, 3p, 14q and 20p) in cohorts with strict genealogic and diagnostic criteria from the Central Valley of Costa Rica (CVCR) and US and Israeli Ashkenazim.

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Fine-mapping of a susceptibility gene locus on 3q for nephropathy in patients with type 1 diabetes. *B. He[1], A.M. Österholm[1], J. Pitkäniemi[2], C. Sarti[2], J. Tuomilehto[2], K. Tryggvason[1].* 1) Matrix Biology, MBB, Karolinska Institute, Stockholm, Sweden; 2) Dept. of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland.

Diabetic nephropathy (DN), the leading cause of end-stage renal disease, is one of the severe complications of type 1 and type 2 diabetes. Almost 30 % of all patients with diabetes develop DN. Clinically, DN manifests with proteinuria and progresses into impaired renal function. Epidemiological studies have provided evidence for genetic factors regulating the development of DN. To map loci linked to DN, we performed a genome scan using Finnish discordant sib pairs (DSPs), sibs affected with type 1 diabetes but discordant for nephropathy. One region on 3q has been identified with a suggestive linkage (LOD score 2.6, P = 0.0004). This region was before reported with significant linkage in American-Caucasians. Therefore, the 3q region most likely harbors a major susceptibility gene for DN. To identify a susceptibility gene for DN, we firstly re-defined a Finnish set consisting of 206 cases (type 1 diabetic nephropathy) and 220 controls (type 1 diabetes alone). In the following step, we carried out case-control studies to test for association by genotyping 55 microsatellite markers across the 64 cM region of the 3q locus in the Finnish case-control set. Two markers showed significant association (P = 0.0007 and 0.003) between cases and controls. After Bonferroni correction, the P value of 0.038 for the most significant marker remained significant. We designate these two marker regions as the LOC1 and 2. We studied LOC1 by genotyping three additional flanking markers. One marker, 50 kb centromeric from the most significant marker, showed significant association (P = 0.037), while the other two markers, 330 and 670 kb telomeric from the most significant marker respectively, did not. Therefore, we narrowed LOC1 to a critical region of 380 kb. LOC1 contains an interesting candidate gene that is expressed in kidney glomeruli. We have sequenced all exons of this candidate gene, but no mutations were shown to be associated with DN. A large-scale SNP genotyping is under way.

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Identification of a novel locus for Myotonic Dystrophy in human chromosome 16. *L.L. Bachinski[1], F. Lehmann-Horn[2], K. Jurkat-Rott[2], J. Gamez[3], S. Raskin[4], R. Krahe[1].* 1) Sect. Cancer Genetics, Dept. Molecular Genetics, Univ. of Texas M.D. Anderson Cancer Center, Houston, TX; 2) Dept. of Applied Physiology, Ulm Univ., Germany; 3) Dept. of Neurology, General Hospital Vall d'Hebron University, Barcelona, Spain; 4) Neurology Service, Dept. of Internal Medicine, Universidade Federal do Parana, Curitiba, Brazil.

Myotonic dystrophy types 1 and 2 (DM1 and DM2) are caused by expansion of $(CTG)_n$ and $(CCTG)_n$ repeats located in *DMPK* (19q13.3) and *ZNF9* (3q21.3), respectively. A third DM locus was mapped to chromosome 15q21-q24, but the mutation is unknown. During the course of a genome scan, one family without expansions in *DMPK* or *ZNF9* and whose disease did not segregate with these loci gave a LOD score of >1.3 in chromosome 16. We genotyped additional markers in this and other families segregating autosomal dominant progressive myotonic myopathy that were also negative for expansions in *DMPK* or *ZNF9*. Using 9 such families (4 German, 4 Spanish and 1 Brazilian), we obtained a LOD score of 3.83 in 16p. Haplotype analysis determined the minimal region shared by these 9 families. Hypothesizing that DM in these families is due to an expanded repeat of the type $(CTG)_n$, $(CCTG)_n$, or other similar sequence, we interrogated all such repeats in the shared region having a length 4. We used amplification across the repeat to check for non-Mendelian inheritance, a hallmark of expanded repeat diseases, and a repeat-primed assay (RP-PCR) to identify expansions. None of the candidate repeats gave evidence of expansion by either RP-PCR or non-Mendelian inheritance. These families are small and each contributes only minimally to the total LOD score. However, in all cases the LODs observed are near the simulated ELODmax. Nevertheless, it is impossible to exclude locus heterogeneity within this set of families, thus complicating localization of the shared region. We conclude that a locus responsible for DM in most of these kindreds maps to chromosome 16, and we are continuing to search for the causative mutation by interrogating repeat sequences in the surrounding regions.

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Genetic Heterogeneity of Cataract at Chromosome 22q. *G. Billingsley[1, 4], S. Santhiya[2], J. Graw[3], A.D. Paterson[1], D. Malaiyandi[1], E. Héon[1, 4].* 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Dr. ALM Postgraduate Institute of Basic Medical Sciences, Department of Genetics, University of Madras, Taramani Chennai, India; 3) GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg, Germany; 4) Dept of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Canada.

Autosomal dominant cataract is a phenotypically and genetically heterogeneous disorder. The chromosomal region 22q11.2-q12, containing the -crystallin gene cluster, has now been associated with a number of congenital cataracts. Mutations in exon 6 of CRYBB1 and CRYBB2 have been associated with pulverulent cataract, congenital nuclear cataract with microcornea and central nuclear cataract. The Q155X mutation in exon 6 of CRYBB2 has marked phenotypic variability producing cerulean, Coppock-like and sutural/cerulean cataracts. Recently a homozygous mutation in exon 6 of CRYBB3 was reported in a family with AR congenital cataract.

We have mapped an autosomal dominant congenital lamellar cataract to the -crystallin gene cluster on chromosome 22q11.2 in a large four generation Indian family with nineteen available members (11 affected and 8 unaffected). All affected individuals had nonprogressive cataract since birth, with partial vision even when not operated. The affected status (presence of cataracts) was determined using slit lamp biomicroscopy or direct ophthalmoscope prior to genetic analysis. Functional candidate genes CRYG (A ->D) and GJA8 were excluded by direct sequencing. Following a genome wide scan (388 microsatellite markers), linkage was detected with marker D22S1167 (LOD score 3.17, = 0). Haplotype analysis with closely flanking markers could not exclude any of the -crystallin genes from the critical disease interval. Sequencing exon 6 of CRYBB1, CRYBB2, and CRYBB3 (previously published to contain mutations) did not reveal any disease-associated mutations in this family. This work further outlines the genetic heterogeneity of congenital cataracts at the 22q locus.

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Autosomal dominant retinitis pigmentosa in a large Belgian family: linkage-based exclusion mapping of 13 known loci. *B.P. Leroy[1,2], K. Robberecht[2], F. Coppieters[1], D. Beysen[1], J. Hellemans[1], P.J. Coucke[1], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium.

Purpose: Linkage-based exclusion mapping of candidate genes for autosomal dominant retinitis pigmentosa (ADRP) in a large Belgian family with ADRP.

Patients and Methods: Genetic material from twenty-four affected and twenty-six unaffected individuals of a 4-generation Belgian ADRP family was available for the study. A clinical diagnosis was made either on the basis of a clinical examination or an extensive questionnaire unequivocally differentiating affected from unaffected family members. Linkage-based exclusion analysis was performed using 26 microsatellites closely linked to 13 currently known ADRP genes (*CA4, CRX, FSCN2, IMPDH1, NRL, PRPF3, PRPF8, PRPF31, RDS, RHO, ROM1, RP1, RP9*).

Results and Conclusion: Segregation analysis of the 26 markers in affected and unaffected family members allowed exclusion of these 13 known ADRP genes. Exclusion of a fourteenth gene (*GUCA1B*) is ongoing, although its causal relationship with ADRP is not clear as yet. The results of our study suggest the involvement of a novel ADRP disease gene as the cause of the phenotype in this family. A genome-wide linkage analysis will be performed to identify this novel locus. Computer simulation of the power of this family predicted significant LOD-scores (> 9) potentially generated from genome-wide linkage analysis.

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Association of KCNJ11 E23K polymorphism with type 2 diabetes in the Saudi population. *O. Alsmadi[1], K. AL-Rubeaan[2], S.M. Wakil[1], F. Imtiaz[1], H. Khalil[1], P. Carroll[1], M. Rajab[1], S. Al-Katari[2], M. Al-Katari[2], B.F. Meyer[1].* 1) Aragene, KFSH&RC, Riyadh, Saudi Arabia; 2) Diabetes Center, King Saud University, Kingdom of Saudi Arabia.

E23K Polymorphism in KCNJ11alters the ATP-potassium channel function of pancreatic beta cells leading to insulin release inhibition, and development of type 2 diabetes. Previous studies indicated that E23K polymorphism is associated with type 2 diabetes in Caucasian populations. Type 2 diabetes pathogenesis is underlined by both polygenic and environmental factors. To date there is no data available on the role or the association of E23K polymorphism with type 2 diabetes in any Arab population. The incidence of type 2 diabetes in Saudi population is substantial bringing high significance to this study. We developed a high throughput molecular beacon based real-time PCR assay for E23K genotyping. We studied a group of 1331 Saudi patients diagnosed with type 2 diabetes based upon WHO criteria, and 219 normal controls (age >60 yr and fasting blood glucose <7mmol/L). The KCNJ11 normal E allele frequencies were 0.80 and 0.89 in type 2 diabetes patients and normal controls respectively. The KCNJ11 risk K allele frequencies were 0.20 and 0.11 in type 2 diabetes patients and normal controls respectively. The difference between the E and K allele frequencies was highly significant comparing type 2 diabetes patients to normal controls ($p = 0.00001$, and OR = 2.02). While this data does not exclude the role of other genes involved in development of type 2 diabetes in Saudi population, it indeed suggests a strong association between E23K and susceptibility to or pathogenesis of type 2 diabetes in the Saudi population.

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Epidemiological and Genetic Aspects of the Metabolic Syndrome in the Family Blood Pressure Program. *A.T. Kraja[1], D.C. Rao[1], A.B. Weder[2], R. Cooper[3], J.D. Curb[4], C.L. Hanis[5], S.T. Turner[6], M.de Andrade[7], C.A. Hsiung[8], T. Quertermous[9], X. Zhu[3], M.A. Province[1].* 1) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 2) University of Michigan Hospitals, Ann Arbor, MI; 3) Loyola University Medical Center, Maywood, IL; 4) Pacific Health Research Institute, Honolulu, HI; 5) Human Genetics Center University of Texas-Houston Health Science Center; 6) Division of Hypertension,; 7) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 8) National Health Research Institutes, Division of Biostatistics, Taipei, Taiwan; 9) Stanford University School of Medicine, Stanford, CA.

Metabolic syndrome (MetS) was investigated in the Family Blood Pressure Program. Information on a total of 11,343 participants was considered in the qualitative MetS (c-MetS). Quantitative MetS (q-MetS) was analyzed in 7,562 participants with no missing values for any of the 10 risk factors. The prevalence of c-MetS as defined by the NCEP criteria, differed by ethnicity. The combined c-MetS prevalence was 37.4% in African Americans and 46.1% in Whites. The percentage of Japanese above the NCEP threshold for WAIST was half that of Hispanics, African Americans, or Whites, but twice that of the Chinese. The percentage of Japanese participants above the NCEP threshold for TG was similar to that in Whites, but about twice as large as in the Chinese. Twice as many Whites were above the TG threshold than African Americans. The prevalence of c-MetS by design in Hispanics was high at 73%. Asians had a c-MetS prevalence of 21.4%. Heritabilities ranged from 66% for Obesity-INS to 11% for BP factor. Linkage analysis detected two major QTLs: one for Obesity-INS with a lod score 3.94, located on chromosome 18p11.21 in GENOA African Americans (D18S53, 41.24 cM), and another for BP with a lod score 3.22, located on chromosome 17q23.1 in Hispanics (D17S1290, 82 cM). These two QTLs, together with 4 additional QTLs with lod scores above 2.5 and 30 additional QTLs with lod score above 1.7, offer hope for dissecting the genetic architecture of MetS with positive implications for molecular diagnosis, prognosis and potential medical intervention.

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APOE allele frequencies and Alzheimers disease risk in the Midwestern Amish population revisited. *J. Virgadamo[1], J.M. van der Walt[1], W.K. Scott[1], S. Slifer[1], P. Gallins[1], P.C. Gaskell[1], E.R. Martin[1], K. Welsh-Bohmer[1], M. Creason[2], A. Crunk[2], D. Fuzzell[2], L. McFarland[2], C.E. Jackson[3], J.L. Haines[2], M.A. Pericake-Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN 37232; 3) Department of Medicine, Scott and White Memorial Hospital, Temple, Texas.

Apolipoprotein E (APOE) has been confirmed as a major risk factor for Alzheimers disease (AD) in multiple populations. Previously, we reported low APOE4 allele frequencies in an Amish pedigree from the Midwest (Adams, Co., IN) and suggested that low prevalence of AD reported in this Amish population may be due to the decreased frequencies of APOE4. In this study, we have expanded our data set to include 9 pedigrees located within three midwestern Amish communities (Adams Co. IN, Elkhart/LaGrange Co., IN and Holmes Co., OH). Frequencies for APOE 2,3,and 4 alleles were computed for the affected (AD + mild cognitive impairment) and unaffected groups in each community. Overall, we observed a significant increase in APOE4 frequency in the affected group (n=114) compared to Amish controls (n=102) (p=0.004). However, when we examined frequencies within each community separately, we observed a significant difference in allele frequency between affected (29.3%) and unaffected (8.82%) groups only in Holmes Co.(p=0.02). Linkage analysis of SNPs indicated significant evidence for linkage only in Holmes Co. with maximum two-point LOD scores of 2.26 for the dominant model and 2.08 for the recessive model. Furthermore, APOE4 allele frequencies were significantly decreased in the Adams Co. unaffected group when compared to a set of outbred Caucasians (p=0.03) which replicates our earlier finding. Thus it appears that a minority of the genetic effect responsible for AD in the Holmes Co. Amish community may be related to APOE but that APOE has little influence on AD in the Adams and Elkhart/LaGrange communities. We are currently conducting a fine mapping study on these pedigrees to identify chromosomal regions that influence AD risk in each community.

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Do variations in genes involved in the potassium-recycling pathway contribute to noise-induced hearing loss? *L. Van Laer[1], P.-I. Carlsson[2,3], A. Raes[4], M.-L. Bondeson[5], A. Vandevelde[1], A. Konings[1], E. Borg[3], D. Snyders[4], G. Van Camp[1].* 1) Dept Med Genet, Univ Antwerp, Belgium; 2) Dept Audiol, Örebro Univ Hosp, Sweden; 3) Ahlsén Res Inst, Örebro Univ Hosp, Sweden; 4) Lab Mol Biophysics, Physiol, Pharmacol, Univ Antwerp, Belgium; 5) Dept Genet Pathol, Uppsala Univ, Sweden.

Noise-Induced Hearing Loss (NIHL) is one of the most important occupational diseases; 400 to 500 million people in the USA and Europe are being exposed to excessive noise on a daily basis. NIHL is a complex disease caused by an interaction between genetic and environmental factors. The various environmental factors involved in NIHL have been relatively extensively studied. On the contrary, little research has been performed on the genetic factors responsible for NIHL. To test the hypothesis that variation in genes involved in potassium recycling in the inner ear might partly explain the variability in susceptibility to noise, we performed an association study using several Single Nucleotide Polymorphisms (SNPs) selected in these genes. Audiometric data from 1261 noise-exposed laborers were examined. DNA was extracted from the 10 % most susceptible and the 10 % most resistant individuals. 23 SNPs selected in 10 genes involved in the potassium-recycling pathway were genotyped. No significant differences were obtained between susceptible and resistant individuals except for 2 SNPs of the KCNE1 gene. One of the associated SNPs was a non-synonymous coding SNP. The possibility that the latter SNP was causative was investigated with electrophysiological studies. The susceptibility variant was introduced into wild-type KCNE1 cDNA and transfected into CHO-cells. Patch-clamp experiments were performed in high K+-concentration to investigate the functional relevance of the KCNE1 variant. The normalized current generated through channels containing the susceptibility variant significantly differed from that through wild-type channels. Furthermore, we obtained a different $V_{1/2}$ for the two types of channels. This putative association still needs to be replicated in other, independent noise-exposed populations.

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Neuropathology of LRRK2 6055G>A (G2019S) Mutation. *O.A. Ross, D.W. Dickson, M.J. Farrer.* Departments of Neuroscience and Pathology, Mayo Clinic, Jacksonville, FL.

In 2002 Funayama and colleagues reported a novel locus, chromosome 12p11.2-Q13.1, for autosomal dominant Parkinsons disease (ADPD; PARK8). Linkage to this region was independently confirmed in a number of kindreds and in November 2004, causal mutations in the novel gene leucine-rich repeat kinase 2 (LRRK2) were identified. A common pathogenic G2019S substitution in the Lrrk2 kinase domain, has been reported to account for ~5% of familial and ~1% of sporadic PD. To date G2019S carriers have presented clinically with typical PD, but only three autopsy reports of mutation carriers, all with typical Lewy body pathology in substantia nigra, have been reported. Our objectives were to determine the frequency of G2019S-positive cases in a large collection of parkinsonism brains. DNA was extracted from over 1500 brains and screened for the (G2019S) mutation with Taqman Probe Chemistry on an ABI7900 sequencer. Positive and negative controls were used. The screened samples included PD (n=405), progressive supranuclear palsy (n=326; PSP) and multiple system atrophy (n=43; MSA). Control groups consisted of brains of clinically normal, aged individuals (n=156), and subjects with a clinical diagnosis of Alzheimers disease (AD; n=654). We identified 10 G2019S-positive cases, with material available for pathological review on eight of the cases. Eight of the ten individuals demonstrated Lewy body pathology, ranging from brainstem predominant to diffuse cortical Lewy body disease. One AD and one control harboring the mutation were identified. This is the first comprehensive screening for G2019S in a large collection of pathologically-confirmed neurodegenerative disorders. The presence of Lewy bodies in 80% of the G2019S cases would indicate that this mutation is responsible for alpha-synuclein pathology. The absence of Lewy pathology in some G2019S carriers may reflect reduced penetrance of the mutation or the pathological pleomorphism that has been described for other Lrrk2 variants. In this context, diverse pathological processes not due to a common mutation raises questions about whether pathology or genetics is the gold standard.

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Patients with eating disorders show increased blood levels of BDNF irrespectively of the Val66Met functional variant. *J. Mercader[1], M. Ribasés[1], M. Gratacòs[1,2], F. Fernandez-Aranda[3], X. Estivill[1,2].* 1) Genes & Disease Program, Ctr Genomic Regulation, Barcelona, Spain; 2) CeGen, National Genotyping Center, Barcelona, Catalonia, Spain; 3) Department of Psychiatry, Hospital Princeps d'Espanya, L'Hospitalet de Llobregat, Barcelona, Catalonia, Spain.

Association of the Val66Met and -270C/T SNPs of the BDNF gene with Eating Disorders (ED) and the results of murine models suggest that increased levels of BDNF in the central nervous system (CNS) could be involved in restricting food intake and low body weight. We have studied if these variants are related to high BDNF levels, which could be contributing to the development of ED. The BDNF levels of 118 ED patients were quantified by ELISA and were analyzed depending on the Val66Met and -270 C/T genotypes. We further compared the levels of 50 of these patients to their 50 unaffected siblings. BDNF levels were higher in the ED patients homozygous for the -270C risk allele (41.0 ng/ml vs 26.9 ng/ml; $p = 0.012$), while we could not detect any effect of the Val66Met variant. Furthermore, plasma BDNF levels were higher in ED patients than in their unaffected sibs (57.7 ng/ml vs. 40.9 ng/ml; $p = 0.004$). Odds ratio were two-fold for the individuals with levels between 31.1 and 58 ng/ml, and ten-fold for individuals presenting levels higher than 58 ng/ml. Finally, a negative correlation between BDNF plasma levels and Global Severity Index and Positive Symptom Distress index of the Symptom Check List (SCL-90R) was found in AN patients. These results provide physiological evidence for a role of this neurotrophic factor in ED, and argue for its involvement in eating behavior and body weight regulation. Ribases et al, 2004; Kernie et al, 2000; Pelleymounter et al, 1995. Supported by FIS-Fondo de Investigaciones Sanitarias and RGPG-FIS (G03/184).

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Strategic Identification and Prioritization of Human Ion Channel Genes as Candidate Neurological Channelopathy Targets. *N. Gupta[1], M. Simoneau[1], J.-F. Poulin[1], K. Boisvert[1], F. Lafreniere[1], M.K. Charles[1], M. Albanese[1], R.G. Lafreniere[1], G.A. Rouleau[1, 2].* 1) Emerillon Therapeutics Inc., Montreal, Quebec, Canada; 2) Universite de Montreal, Montreal, Quebec, Canada.

Ion channel dysfunction is the basis of many episodic neurological disorders. To identify relevant drug targets for such diseases, we have initiated a high throughput mutation detection screen of 150 brain-expressed human ion channel genes using a panel of 368 DNA samples from unrelated patients with epilepsy, Tourette syndrome (TS), bipolar affective disorder (BD), essential tremor (ET) or migraine. The first phase of this project was to compile a systematic list of all the known human ion channel genes, which was done by querying publicly available human genome assemblies through websites such as the UCSC Genome Browser, Ensembl, NCBI and OMIM. The list also took into account comprehensive reviews on ion channels or channelopathies. Finally, homology searches were conducted to identify paralogs or pseudogenes of each of the genes. A list consisting of 547 human ion channel genes and 11 pseudogenes was assembled, which included all major families of ion channels. Of these, 468 genes have been further analyzed through literature and database mining to determine: i) expression pattern, ii) subcellular localization, iii) physical proximity to a region linked through genetic studies to any of the disorders, iv) implication in a given human disease, and v) effect of null mutations in animal models. An algorithm was developed to take into consideration these various criteria and calculate a prioritization score for each gene. Genes previously identified as being involved in epilepsy, such as SCN1A and CLCN2, scored high in our selection scheme, validating our candidate gene selection criteria. So far we have prioritized 138 ion channel genes and are screening them in our panel of samples. Identification of causative mutations in ion channel genes will help the diagnosis and treatment of such inherited neurological epoisodic disorders.

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SAGE Expression Analysis of Trabecular Meshwork to Identify POAG Susceptibility Genes. *M. Hauser[1], D. Layfield[1], J. Yang[1], T. Wang[1], E. Hoffman[3], D. Stamer[3], R. Allingham[2].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Dept of Ophthamology, Univ of Arizona, Tuczon, AZ; 3) Dept of Ophthamology, Duke Univ Medical Ctr, Durham, NC.

Elevated intraocular pressure is the leading risk factor for primary open angle glaucoma. We hypothesize that gene expression changes in the trabecular meshwork (TM) will reveal determinants of fluid flow facility and thus the mechanism of this IOP elevation. We have profiled gene expression in the TM to shed light on the metabolic activity of this tissue and to identify candidate susceptibility genes for POAG. Serial Analysis of Gene Expression (SAGE) was performed on human TM tissue. Candidate genes were sequenced in 16 POAG patients and controls. Taqman was used to genotype single nucleotide polymorphisms for association analysis. Among the most abundantly expressed genes in the TM is Cathepsin S, which degrades extracellular elastic lamina and could lead to increase resistance to fluid flow in the TM. The fluid transporters Aquaporin 3 and 5 are also expressed in the TM, as are over 50 genes involved in cell-cell adhesion that could influence fluid flow facility. The SAGE data also reveal alternative splicing of transcripts. For example, there are two common splice forms of the serine arginine-rich pre-mRNA splicing factor (SR-A1), but only one of these forms is expressed in the TM. We have combined these SAGE expression profiles with our existing genomic linkage data for POAG (HMG 9: 1109). Those four linkage regions contain 1065 UNIGENE clusters, but only 118 of these genes are expressed in the TM, and even fewer are also differentially expressed in patients and controls. Because they are both positional and functional candidates, these genes are prioritized for analysis in POAG patients and controls. High throughput SNP association and gene sequencing is underway for these candidates. To date no variants associated with POAG have been found in NDN (Necdin homolog), NDNL2 (Necdin-like 2), and the receptors GABRA5, B3, and G3.

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A new web tool: the Italian Bank of Rett Syndrome. *F. Ariani, I. Meloni, C. Pescucci, K. Sampieri, I. Longo, F. Mari, M. Bruttini, E. Scala, R. Caselli, C. Speciale, A. Renieri.* Dept Mol Genet, Med Genet, University of Siena, Siena, Italy.

Rett syndrome (RTT) is a progressive neurodevelopmental disorder almost exclusively affecting females. MECP2 mutations are found in 70-80% of cases. Recently, CDKL5 mutations have been found associated with the early onset seizure variant of RTT. The genetic alteration in the remaining cases is unknown. In order to favour the clarification of the pathogenic mechanisms of RTT we have created a bank collecting a large panel of patients. Researchers can access to the bank from the introductory page available at http://www.bank.unisi.it. On the main page users can see a list of all patients available with additional specific information. At present the site contains 187 probands and their relatives. In 137 probands a pathogenic mutation has been identified. The database is organized on two levels: a public level freely available to the general public and an administrator level accessible only to bank administrators through the use of a username and password and containing personal data of patients and relatives and detailed clinical information. In both cases, the main page of the site consists in a table containing a list of all samples available. The following information is available for each sample: a) CODE and INTERNAL CODE (a progressive number which identifies the single families and family members); b) PHENOTYPE (classic, variants); c) MUTATED GENE NAME; d) MUTATION TYPE; e) NUCLEOTIDE and AMINOACID CHANGE; f) ADDITIONAL INFO; g) AVAILABLE BIOLOGICAL SAMPLES; h) PUBMED REFERENCE. In addition users can rapidly find the patients of interest by exploiting the Search by option allowing users to select patients by: i- Mutated gene; ii- Mutation type; iii- Nucleotide change; iv- Aminoacid change; v- Phenotype. Finally, on the main page of the bank, users can find links to three additional pages: a) List of mutations; b) Graphic of mutations; c) Non pathogenic rare variants. This bank collecting a large number of patients will represent an important tool for all researchers working on RTT.

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Investigation of TNF cleavage enzyme (TACE) gene polymorphism with rheumatoid arthritis. *C. Potter, J. Worthington, A. Barton.* ARC-EU, The University of Manchester, Manchester, United Kingdom.

Background: The tumour necrosis factor alpha (TNF) cleavage enzyme (*TACE*) is an important regulator of the pro-inflammatory cytokine TNF. It, therefore, represents a strong candidate susceptibility gene for diseases such as rheumatoid arthritis (RA) in which inflammation is mediated by TNF. Methods: 18 SNPs spanning the *TACE* gene were genotyped (Taqman, ABI, UK) in a test cohort comprising 96 Caucasian RA affected sibling pairs (ASP) (192 cases) and 183 Caucasian unrelated controls. Associations were evaluated using a method that accounts for the relatedness of cases (1). Any SNPs showing association ($p<0.05$) were genotyped in a larger replication cohort of 891 unrelated cases and 547 unrelated controls. In addition, haplotype-tagging (ht) SNPs defining the surrounding region were identified from the HapMap data (www.hapmap.org) and genotyped in a 3rd cohort of 359 unrelated cases and 368 controls using RealSNP assays (Sequenom Inc, Cambridge, UK). Haplotype and linkage disequilibrium analysis were undertaken using HelixTree software (Golden Helix, USA). Results: One SNP (rs11684747), mapping to the 5' region of the *TACE* gene, was significantly associated with RA ($p=0.0087$) in the test cohort, with cases being 1.6 and 3.9 times more likely to carry a single or two copies of the minor G allele, respectively (OR AG genotype = 1.6, 95% CI 1.0-2.6, $p=0.04$ and OR GG genotype = 3.9, 95% CI of 1.1-16.6, $p=0.015$). However, this finding was not observed in the replication cohort (OR GG genotype = 0.8, 95% CI 0.4-1.5, $p = 0.4$). In addition, no difference in haplotype frequencies was observed for the promoter htSNPs when compared in the 3rd cohort. Conclusion: The initial association to a promoter polymorphism was not replicated in other cohorts of RA patients and is likely to represent a false positive finding in the test group. Through a combination of random SNPs and htSNPs, the *TACE* gene has been thoroughly screened for association to RA. Therefore, we conclude that polymorphism within the *TACE* gene is unlikely to play a major role in determining susceptibility to RA. References [1] Slager and Schaid, Am J Hum Genet. 2001.

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Variation in the complement C5 gene is not associated with rheumatoid arthritis. *K.B.S. Spreckley, S. Eyre, J. Bowes, J. Worthington, A. Barton.* University Of Manchester, ARC Epidemiology Department, Stopford Building, Oxford Road, Manchester. M13 9PT.

Background: Complement 5 (C5) is a key mediator of the inflammatory response and has been implicated in several autoimmune diseases including rheumatoid arthritis, RA. The collagen induced arthritis model of RA has shown that the *C5* gene is involved in the regulation of the inflammatory response. Hence, the *C5* gene is a strong candidate susceptibility gene for RA.

Aim: To investigate association of the *C5* gene with RA.

Methods: In a screening cohort of 192 UK Caucasian RA cases and 180 controls, 19 single nucleotide polymorphisms (SNPs) spanning the *C5* gene were tested for association with RA. Haplotype tagging SNPs were preferentially selected using Hapmap (http://www.hapmap.org/) and Perlegen (http://www.perlegen.com/) to ensure total coverage of the gene. Genotyping was performed using the Sequenom mass spectrometry platform. Haplotypes were estimated using the EM algorithm executed in Helix Tree (http://www.goldenhelix.com) and genotype, allele and haplotype frequencies were compared between RA cases and controls.

Results: The median inter marker spacing of the SNPs was 3.2 Kb. One SNP (rs17292882) deviated from Hardy Weinberg expectations ($p<0.01$) and was eliminated from subsequent analysis. For the remaining 18 SNPs no difference in allele, genotype or haplotype frequencies were observed between RA cases and controls. Given the sample size and allele frequencies of the SNPs tested, association of the gene with RA was excluded for modest effect sizes.

Conclusion: Data from this study provides no evidence for the involvement of the *C5* gene in determining susceptibility to RA. However, the C5 pathway appears crucial to the inflammatory process. Interestingly, C5a-receptor (C5aR) knockout mice are resistant to induction of CIA suggesting that polymorphisms within the *C5aR* gene may play a role in RA disease susceptibility, but this requires further investigation.

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Evaluation of the human *LITAF*(*SIMPLE*) gene as a candidate gene for rheumatoid arthritis in two distinct Caucasian populations. *S.K. Hall[1], M. Man[3], Y.-T. Fan[2], B. Dalziel[4], A.B. Seymour[1], J. Mobley[5], D.P. King[1].* 1) Discovery Pharmacogenomics; 2) Non-Clinical Biostatistics; Pfizer Global Research & Development, Groton, CT 06340; 3) Non-Clinical Biostatistics; 4) Clinical Pharmacogenomics; 5) Inflammation Pharmacology, Pfizer Global Research & Development; Ann Arbor, MI 48105.

Rheumatoid arthritis is a chronic inflammatory disease with multiple systemic features in addition to joint destruction. Patients with rheumatoid arthritis are characterized by excessive TNF- production and, although some controversy exists regarding its predicted amino acid sequence, the human *LITAF* gene (also referred to as *SIMPLE*) has been reported to play a role in the regulation of TNF- expression, inhibition of which has demonstrated therapeutic effects in patients with rheumatoid arthritis. In this study, we sought to determine whether genetic variation in *LITAF* is associated with susceptibility to rheumatoid arthritis using a case control study design. In our initial population, 10 single nucleotide polymorphisms in the *LITAF* gene were genotyped in 133 Caucasian subjects with mild to moderate rheumatoid arthritis and 346 ethnically matched control subjects, with no personal history of RA or first-degree relatives with the disease. Potential control subjects were also screened for Rheumatoid Factor and C-reactive Protein (CRP) and excluded if positive. These data were analyzed using a generalized linear model, including covariate terms for ethnicity, sex, and age. A preliminary significant association was identified for SNP rs2868424 in the first intron of the *LITAF* locus (p0.01). Based on these findings, a second study was performed using 570 rheumatoid arthritis subjects and 570 matched controls in an attempt to replicate the initial positive association with SNP rs2868424. However, no significant association was observed for this SNP and rheumatoid arthritis in this population. These data provide some suggestive evidence for a role of *LITAF* in rheumatoid arthritis but, taken together, do not provide consistent genetic evidence for its involvement in this disease.

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Association studies of two functional *CTLA4* polymorphisms with Juvenile Rheumatoid Arthritis. *S. Prahalad[1], M. Honeggar[2], S.D. Thompson[3], D.N. Glass[3], J.F. Bohnsack[1], M.J. Bamshad[1,2].* 1) Dept Pediatrics, Univ Utah Sch Medicine, Salt Lake City, UT; 2) Eccles Institute of Human Genetics, Univ of Utah, Salt Lake City, UT; 3) Cincinnati Children's Hosp Med Center, Cincinnati,OH.

Cytotoxic T-lymphocyte Antigen 4 (CTLA4), expressed on the T-cell surface, plays a negative role in regulating T-cell activation. Single nucleotide polymorphisms (SNPs) in *CTLA4* have been associated with susceptibility to several T-cell mediated autoimmune disorders including type 1 diabetes, rheumatoid arthritis, and thyroiditis. A SNP in the promoter region of *CTLA4* has been associated with juvenile rheumatoid arthritis (JRA), a disorder in which activated T-cells predominate the inflamed synovium. To test the hypothesis that variants in *CTLA4* will be associated with susceptibility to JRA or subtypes of JRA, 340 affected children with JRA (138 pauci, 114 poly and 88 systemic onset) and their families were genotyped for SNPs located in the promoter region (-318 C/T) and in exon 1 (49A/G) of *CTLA4*, by PCR amplification and digestion with Mse1 and Tse1, respectively. SNPs were tested for association with JRA by transmission disequilibrium testing using Transmit 2.5.4. Analyses were repeated after stratification by onset-type, age of onset, and course. No significant deviation of transmission of either the -318C/T SNP or the 49A/G SNP to the children with JRA was found. Furthermore, neither SNP showed any deviation of transmission- after stratification by onset type, onset age (early <6 years or late onset > 6 years), or course (pauciarticular or polyarticular course). A second independent cohort of 120 children with JRA (61 pauci, 59 poly) and 180 unrelated ethnicity-matched controls were similarly genotyped for case-control analyses of these two SNPs. There were no significant associations between genotypes or alleles of either SNP with JRA or subtypes. We were unable to replicate the association of the -318C/T SNP previously reported in children of German ancestry with JRA, in two cohorts of children with JRA from the U.S. Our results suggest that these two functional *CTLA4* variants, implicated in numerous autoimmune disorders, are not associated with susceptibility to JRA or subtypes of JRA.

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Is the LRP1/A2MR gene expression indicative for degenerative cell metabolism and disease? *C. Glaeser[1], G. Birkenmeier[2], S. Schulz[1], K. Huse[3].* 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Inst Biochemistry, Univ Leipzig, Leipzig, Germany; 3) Inst Molec Biotechnology, Jena, Germany.

The final state of degenerative diseases like Alzheimer's disease (AD) or coronary artery disease (CAD) can be described as a result of disordered cellular metabolism characterized by a disordered supply and disposal system. One hallmark of these degenerative processes is the LDL receptor-related protein 1 (LRP1/A2MR) which functions as a multi-ligand cargo-receptor for several components like A2M, ApoE, and APP. Patients: We performed a comparative study about the in vivo LRP1 gene expression in 3 groups of probands, all from Germany and of Caucasian origin. The 1st group consisting of 9 individuals with Alzheimer's disease (AD) (mean age 71y, SD 8.62; 4 males), the 2nd group consisting of 68 patients with coronary artery disease (CAD) (mean age 58.43y, SD 4.09; 49 males) and the 3rd group with 20 clinically normal individuals (58.9y, SD 3.39; 18 males) serving as control. Methods: LRP1 gene expression was measured in freshly isolated monocytes from venous blood by real-time RT-PCR procedure. Results: The expression level in monocytes of the three groups of probands differed significantly from each other (p=0.015). The highest LRP1 expression level was observed in AD patients (290.96 ag/cell) followed by CAD patients (174.49 ag/cell) and by the lowest expression level in monocytes from controls (158.79 ag/cell). There was no significant correlation between age or gender with LRP1 expression. The results demonstrate a disease-related increase of LRP1 gene expression level in monocytes which might serve as a diagnostic feature for AD and CAD.

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Role of homo- and heterozygous PINK1 mutations in a large German family with Parkinson's disease. *K. Hedrich[1], J. Hagenah[1], A. Djarmati[1], A. Hiller[1], C. Schneider-Gold[2], P. Vieregge[3], A. Muenchau[4], C. Klein[1].* 1) Depts Neurology and Human Genetics, Univ Luebeck, Germany; 2) Dept Neurology, Univ Goettingen, Germany; 3) Dept Neurology, Hospital Lippe-Lemgo, Lemgo, Germany; 4) Dept Neurology, University Medical Center Hamburg Eppendorf, Hamburg, Germany.

Background: While homozygous mutations in the PINK1 gene have been unequivocally associated with Parkinson's disease (PD), the role of single heterozygous PINK1 mutations is less clear. **Patients and methods:** After obtaining informed consent, 153 mostly early-onset PD patients underwent a standardized neurological examination. In addition, 19 family members of a homozygous German mutation carrier were included (Family W). We screened for PINK1 mutations by dHPLC (Exons 2-8) and SSCP (Exon 1) analyses, followed by sequencing in cases with a conspicuous elution/band pattern. We also performed gene dosage studies in PINK1 by quantitative duplex PCR. **Results:** We identified two German PINK1 mutation carriers (1.3%), one homozygous (Case 1; index patient of Family W; c.1366C>T; p.Gln456X) and one heterozygous (Case 2; c. 952A>T; p. Met318Leu) patient. Case 1 presented with a phenotype closely resembling idiopathic PD with a late onset, Case 2 had an early onset and additional signs. Family W comprised a total of four affected homozygous (age: 60-71 yrs., age of onset: 47-61 yrs.), seven slightly or mildly affected heterozygous (age: 31-49 yrs.), and four unaffected heterozygous mutation carriers (age: 34-44 yrs.). None of the heterozygous affected family members was aware of their symptoms (asymptomatic). All symptomatic members in Family W had a very benign clinical course and responded extremely well to small doses of L-Dopa. **Conclusions:** Our study suggests that a small but considerable percentage of PD patients carries PINK1 mutations. Heterozygous PINK1 mutations may predispose to PD, as was previously suggested by the presence of dopamine hypometabolism in asymptomatic mutation carriers. Long-term follow-up of our large family provides an excellent opportunity to further evaluate the role of single heterozygous PINK1 mutations later in live, which will have major implications on genetic counseling.

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Familial multiple slerosis is not associated with eIF2B5 gene mutation in Southern Italy. *C. Ungaro, M. Liguori, FL. Conforti, T. Sprovieri, A. Magariello, A. Patitucci, AL. Gabriele, M. Muglia, R. Mazzei.* ISN-CNR, Mangone, CS, Italy.

A lot of evidence has provided that genetic factors contribute to the familial Multiple Sclerosis (MS) susceptibility, a chronic inflammatory demyelination of the central nervous system (CNS). On the basis of the absolute risk of MS in a first-degree relative of a patient is 20-40 times the risk in the general population, an autosomal recessive model of inheritance of MS candidate gene has been suggested. Up to date, the only MS locus has been mapped to the Major Histocompatibility Complex (MHC) superlocus on chromosome 6p21.3. Other genetic elements located within the Human Leukocyte Antigen (HLA) Complex, i.e., Heat Shock proteins (HSPs) have been shown to be strikingly elevated in MS lesions, thus suggesting their role in the pathophysiology of MS. The eukaryotic translation initiation factor (eIF2B), encoded by different genes (eIF2B1-5) is an important factor in preventing the synthesis of denatured proteins during cellular stress and whose function seem to work in parallel with HSPs. Mutations in this gene inherited as autosomal recessive trait cause a stress-sensitive leukoencephalopathy called Vanishing White Matter Disease (VWM) (OMIM 603896). Previous studies have reported cases of VWM with MR images similar to that observed in MS. In the present study we performed the eIF2B5 gene mutational screening in 11 italian pairs of siblings affected by MS. The molecular analysis of the eIF2B5 gene was carried out by Denaturing High Performance Liquid Chromatography (DHPLC) and direct sequencing. We did not find any mutations in the coding regions in both patients and controls, except for a common variation in exon 13 (Ile587Val) in the heterozygous state in 6 MS patients and in the homozygous state in only one MS patient. The same variation was also found in the heterozygous state in 3 out of 10 healthy controls. Therefore, the current data demonstrate that the eIF2B gene is not involved in the development of familial MS and that VWM and familial MS are distinct diseases.

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Eating disorders and related psychopathological traits are associated with serotonin receptor 5HT2C alleles. *M. Ribases[1], J.M. Mercader[1], M. Gratacos[1], M. Morell[1], A. Badia[2], L. Jimenez[2], R. Solano[2], F. Fernandez-Aranda[2], X. Estivill[1].* 1) Genes and Disease Program, Center for, Genomics regulation, Barcelona, Catalonia, Spain; 2) Psychiatric Service, Ciutat Sanitaria Bellvitge, LHospitalet, Catalonia, Spain.

Several lines of investigation support a serotoninergic participation in eating behaviour and body weight regulation and suggest its involvement in the aetiology of eating disorders (ED) and some related psychopathological traits. to test this hypothesis we have analyzed the -995G/A, -759C/T, -697G/C and Cys23Ser SNPs within the serotonin receptor 5HT2C gene by a population-based association study in a total sample of 151 ED patients and 116 sex-matched unrelated controls. We also analyzed the potential involvement of the 5HT2C gene in different psychiatric symptoms measured by the Symptom Checklist 90-revised (SCL90-R) questionnaire. The case-control study showed that the -995G/-759C/-697C/Ser23 haplotype was associated to the purging ED categories of and binge-eating/purging anorexia and bulimia nervosa (P = 0.01). We also observed that bulimic patients carrying the -995A/-759T/-697C/Cys23 5HT2C haplotype showed increased symptomatology for seven of the nine subscales of the SCL90R questionnaire. These include somatization (p = 0.029), obsessive-compulsiveness (p = 0.021), depression (p = 0.032), anxiety (p = 0.004), hostility (p = 0.028), phobic anxiety (p = 0.029) and paranoid ideation (p = 0.008). The results presented here suggest that the 5HT2C gene may participate in the physiopathology of ED, not only through its direct effect on eating behaviour, but also on the anxiety and depressive traits associated to anorexia and bulimia nervosa. Supported by the Fondo de Investigaciones Sanitarias (PI040632 and Psychiatry Genetics network, G03/184), and the Departament dUniversitats i Societat de la informació, Generalitat de Catalunya.

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Several BDNF SNPs, but not the Val66Met functional variant, show association with panic disorder. *M. Gratacos[1,2], R. Martín-Santos[3,4], R. Navinés[3,4], M. Bayés[1,2], R. de Cid[1,2], M. Cabré[4], A. Puig[2], X. Estivill[1,2].* 1) Genes & Disease Program, Center for Genomic Regulation, Barcelona, Catalonia, Spain; 2) Spanish Genotyping Network (CeGen), Barcelona, Catalonia, Spain; 3) Psychiatric Genetics, Pharmacologic Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Catalonia, Spain; 4) Department of Drug Abuse and Psychiatry (IAPS), Hospital del Mar, Barcelona, Catalonia, Spain.

Different studies point to brain-derived neurotrophic factor (BDNF) as involved in the pathophysiology of mood disorders. Several studies have evaluated the functional BDNF Val66Met variant with opposing results. The Val66 allele has been associated with bipolar disorder in Caucasians but not in Asians, a haplotype incorporating Met66 appeared to be protective against bipolar disorder or obsessive-compulsive disorder and, finally, the Met66 allele is strongly associated with anorexia and bulimia. With respect to panic disorder (PD), a recent study has found that genotype and allele frequencies for the BDNF gene Val66Met variant did not differ between PD patients and controls in a group of Chinese patients. Methods: To study the association between BDNF and PD, we performed a casecontrol study in a total sample of 198 PD outpatients diagnosed by SCID-DSM-IV and 178 unrelated sex-matched controls with 30 SNPs covering the entire BDNF gene and using the SNPlex technology. Results: Significant differences in genotype frequencies between PD patients and controls were observed for four BDNF SNPs, but no with the Val66Met variant. Haploview defined two different LD blocs and a haplotype associated to the disease (p=0.04). Conclusions: This study defines BDNF as a gene involved in the susceptibility to PD and reinforces the view that variants in BDNF play a role in different psychiatric disorders that may share common underlying biological features. References: Ribasés et al. (2004) Lam et al. (2004). Support provided by Marato-TV3: (014330) and by Red de Genotipación y Psiquiatría Genética FIS(G03/184).

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Profiling cytogenetic abnormalities in autism using array CGH. *L.A. McInnes, L. Edelmann.* Dept Psychiatry/Human Gen, Mount Sinai Sch Medicine, New York, NY.

Introduction: The advent of array comparative genomic hybridization (array CGH), among other methods, has permitted genome-wide investigation of large genomic duplications and deletions ranging from 1 kb to several hundred kb. Surprisingly, more than one-half of these polymorphic dups/dels have been found to overlap with known genes and likely contribute substantially to human phenotypic variation. We are interested in the role these dups/dels might play in autism, especially as sex ratios of affecteds indicate that autism with mental retardation and dysmorphology may be far less heritable than autism without those features. Autism is associated with at least 8 microduplication/deletion syndromes and we suspect that there will be many more. For instance, recent array CGH profiling studies of children with MR and learning disability show that 10-20 percent of these individuals have microdups/dels that appear to be responsible for the phenotype. Therefore, we screened 20 cases with autism and severe MR from the isolated population of the Central Valley of Costa Rica (CVCR) to look for known and novel microdups/dels associated with autism.

Results: We found 9 unique, novel microdups/dels that may be pathological. We also found one duplication of a clone in 15q12, the Prader-Willi/Angelman region. We also found 3 novel dups/dels in more than one individual including 22q12, 4q32 and 7q21. The latter deletion is of greatest interest as this clone lies immediately next to the breakpoint of a chromosomal rearrangement previously identified in a case of autism.

ConclusionWe will go on to map the break-points of these microdups/dels in order to find potential autism susceptibility genes. Interestingly, Sharp et al. 2005 recently noted that they saw virtually no polymorphic dups/dels that were exclusive to any population in the 4 major population groups they tested. We will continue our study in the CVCR on a larger scale to see if this observation holds true.

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Human BDNF/BDNFOS sense and antisense genes and association study of their genomic polymorphisms with substance abuse vulnerability. *Q.R. Liu, T. Drgon, D. Walther, G.R. Uhl.* Molecular Neurobiology Branch, NIDA/NIH, Baltimore, MD.

Molecular and genetic studies in human have identified brain-derived neurotrophic factor (BDNF) as a candidate locus for involvement in addictions. Human BDNF gene is transcribed from at least 7 promoters and each of the seven alternative exons is independently spliced to a main coding exon. We discovered a novel alternatively spliced natural antisense non-coding gene termed ¡BDNFOS¡ that is transcribed from the opposite strand, using a single promoter. The BDNFOS 225 bp fifth exon is complementary to BDNF's main protein coding exon (exon VIII). The human BDNFOS gene is conserved in chimpanzee but not found in other nonprimate mammalian genomes searched. We have identified novel SSLP and SNP markers within BDNF/BDNFOS genes, several with remarkably- different frequencies in members of different ethnic groups. Allele 228 of the SSLP ss13534842, located at the 5' flanking sequence of the BDNF main coding exon, displayed significant association with drug abuse vulnerability of NIDA European-American samples. The Met66/rs6265 SNP was associated with reduced vulnerability in both African- and European-American samples. BDNF genomic variants remain candidates for modest contributions to human addiction vulnerability (Support NIDA-IRP).

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THE HOLOPROSENCEPHALY-LIKE PHENOTYPE - CHALLENGES IN DIAGNOSIS AND GENETIC COUNSELING. *L. Ribeiro, A. Richieri-Costa.* Hosp Rehab Craniof Anomalies, Univ Sao Paulo, Sao Paulo, Brazil.

Mutations involving the SHH, SIX3, GLI2 and TGIF genes result in a wide phenotypic variability, ranging from normal phenotype to typical holoprosencephaly. We focused our attention in a cluster of 22 patients with normal neuropsychological development and with facial appearance within the so called Holoprosencephaly-like phenotype which have been previously screened for SHH, SIX3, TGIF and GLI2. The main objective of the present study was to observe the evolution of the phenotype and to compare with the type of observed mutation, when present. From this sample, 5 patients showed mutations in the SHH gene, and 1 in the GLI2, the remainder patients showed no mutations in the genes screened. In these patients, several clinical signs are associated randomly and the definition of a heterogeneous condition such as the HPE not always is a simple task. Molecular assays have been mandatory, since 27% of these patients have shown different mutations in SHH or in other genes within the SHH cascade. When we compare the present sample with Mulikens patients there are enough similarities to conclude that we are dealing with the same condition, especially when we consider the changing phenotype with age, moreover, the assumption that most of the patients within the HPE spectrum present neuropsychological delay should be regarded with caution.

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Cross-species micro array analysis identifies Prolactin as a candidate hypothalamic signaling molecule for sexual orientation in sheep. *S. Bocklandt[1], C.E. Roselli[2], E. Vilain[1].* 1) Dept Human Genetics, Sch Med, Univ California, Los Angeles, Los Angeles, CA; 2) Dept. Physiology & Pharmacology, Oregon Health & Science University, Portland, OR.

Sheep are a unique animal model in which to study the hormonal, developmental, and genetic contributions to sexual partner preference. Most domestic rams are sexually attracted to and active with estrous ewes and are referred to as female oriented rams (FORs). However, 8-10% of rams exhibit a sexual partner preference for other males classifying them as male oriented rams (MORs). An ovine sexually dimorphic nucleus (oSDN) was identified in the Medial Preoptic Area of the Anterior Hypothalamus (MPOA), and this nucleus was found to be larger in FORs than in MORs and similar in size in MORs and ewes. We performed a cross-species micro array expression analysis using human spotted cDNA arrays on RNA from the anterior hypothalamus and amygdala of 4 MORs, 3 FORs and 3 ewes in the luteal phase of the estrous cycle, during the breeding season. Using a stringent spot quality filter, 3347 unique genes were selected for analysis. The ovine Prolactin (Prl) transcript showed the most consistent expression difference between the MORs and the FORs, both in the amygdala and the anterior hypothalamus. Quantitative real-time PCR performed on the same subjects showed that Prl was expressed 20.3 times higher in the anterior hypothalamus, and 6.1 times higher in the amygdala, however, no expression differences were found in the frontal cortex or in the pituitary. Furthermore, circulating Prolactin levels were not different between the MORs and FORs. The differences in hypothalamic and amygdala Prolactin expression were not found in animals sacrificed outside the breeding season. We hypothesize that the Prolactin signaling pathway plays a role in the regulation of sexual orientation in sheep. The extremely high prolactin mRNA levels observed in male oriented rams indicate a dysregulation of Prl expression in the hypothalamus and amygdala. It may reflect an unusual expression during brain development, creating a female-typical expression level that would orient rams to a female-typical partner preference.

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Loss of endogenous AR accelerates motor neuron dysfunction and androgen insensitivity in a mouse model of spinal and bulbar muscular atrophy. *P.S. Thomas Jr[1], G. Fraley[2], L. Woodke[3], V. Damian[1], B.L. Sopher[1], S. Plymate[3], A.R. La Spada[1].* 1) Lab Medicine, Univ Washington, Seattle, WA; 2) Biology, Hope College, Holland, MI; 3) Medicine, Univ Washington, Seattle, WA.

X-linked spinal and bulbar muscular atrophy (SBMA) is a slowly progressive motor neuronopathy that also results in mild androgen insensitivity. Evidence from SBMA and other polyglutamine (polyQ) repeat diseases suggests that polyQ expansion causes pathology by imparting a toxic gain of function. However, androgen insensitivity reflects a deficit of wild-type AR function, suggesting that the androgen insensitivity of SBMA involves loss of function as well. In order to investigate the role of normal AR function in SBMA, we compared male mice carrying the human AR with 100 polyglutamine repeats (AR100) to mice carrying the same transgene, but lacking the endogenous Ar (AR100-tfm). Both AR100 and AR100-tfm mice express the same amount of polyQ-expanded AR; however, AR100-tfm mice exhibit earlier onset of weight loss, kyphosis, and hindlimb atrophy, and performed slightly worse on the grip-strength test than did AR100 mice. In addition to the neuromuscular phenotype, absence of Ar uncovered signs of androgen insensitivity. The anogenital distance of male AR100-tfm mice is indistinguishable from females, whereas the anogenital distance of male AR100 mice is normal. AR100-tfm mice display elevated levels of luteinizing hormone (LH) ($p < .01$) and an increased androgen insensitivity index (LH x Free Testosterone); however, testes size in AR100-tfm mice was markedly diminished ($p < .05$). Furthermore, the size of the spinal nucleus of the bulbocavernosis (SNB), which is extremely sensitive to androgen function, is markedly reduced in AR100-tfm mice ($p < .05$). We thus hypothesized that the androgen insensitivity results from a diminished ability of polyQ-expanded AR to activate transcription. Reporter assays using AR-null PC-3 cells revealed a substantial decrease in the transactivation ability of polyQ-expanded AR ($p < .05$). These results suggest that the endogenous Ar ameliorates pathology in a mouse model of SBMA, and supports a role for the loss of wild-type function of AR in SBMA pathogenesis.

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Genome-wide Scan for Autism in an Extended Pedigree from a Regional Subisolate in Finland. *T. Ylisaukko-oja[1,2], T. Varilo[1], H. Kilpinen[1], R. Alen[3], R. Vanhala[4], E. Kempas[1], M. Elmohandess[1], L. von Wendt[4], I. Järvelä[2,5], L. Peltonen[1,2].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Department of Child Neurology, Central Hospital of Central Finland, Jyväskylä, Finland; 4) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 5) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland.

As a part of the study of autism spectrum disorders (ASDs) in the Finnish population, we performed an extensive genealogical search and identified common ancestors for a set of study families. We were able to connect 21 Finnish families with ASDs (n_affected = 31) by genealogical links extending to 17th century. Although the earlier work in the Finnish population has revealed some interesting loci (Auranen et al. 2002; Ylisaukko-oja et al. 2004), the benefits of this isolated pedigree have not been maximally utilized so far. Therefore, we have carried out a dense genome-wide screen in this unique sample using 1100 microsatellites, which yields an average intermarker spacing of 4 cM. Joint analysis of linkage and linkage disequilibrium was performed using Pseudomarker statistics. The best evidence for joint linkage and LD emerged for loci at 1q23 (p=0.0008) and 15q12 (p=0.0008). 1q23 has been among the best loci also in the two earlier genome-wide scans in the Finnish sample (Auranen et al. 2002; Ylisaukko-oja et al. 2004). Furthermore, it is of interest that cytogenetic abnormalities at 15q11-13 are reported to be frequently present in association with autism. Fine mapping of the identified loci and analyses of positional candidate genes are performed in an extended sample of 100 Finnish autism families. The candidates selected for the follow-up analyses include ATP1A2, ATP1A4, KCNJ9, KCNJ10, RGS4 and CAPON at 1q23 as well as UBE3A, ATP10C and GABAA receptor cluster at 15q12.

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An association analysis of candidate genes in the Chromosome 15 q 11-13 (Prader-Willi/Angelman Syndrome Critical Region) and Autism Spectrum Disorder. *S. Curran[1,2], J. Powell[3], B. Neale[2], K. Dworzynski[2], T. Li[2], S. Thomas[4], J. Brown[5], M. Veltman[6], S. Roberts[4], D.G.M. Murphy[1], P. Sam[1,2], P.F. Bolton[2].* 1) Psychological Med, Inst Psychiatry, London, United Kingdom; 2) MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London SE5 8AF; 3) Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF; 4) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire, U.K; 5) Paediatric Medical Unit, Southampton General Hospital, Southampton, UK; 6) Section of Developmental Psychiatry, University of Cambridge.

Autism spectrum disorder (ASD) is a spectrum of related diagnostic categories, comprising subtypes: autism, atypical autism, Aspergers syndrome and other pervasive developmental disorders (ICD-10). Chromosome 15q11-q13 has been identified as a strong candidate region on the basis of autistic symptoms in patients with chromosomal abnormalities in that region as outlined in Bolton et al. (2004) and numerous suggestive linkage and association findings (see Spence, 2004). We have tested 148 simplex U.K. families, characterised by both ADI-R and ADOS to have an autism spectrum disorder (ASD), for association to the GABRB3 gene on chromosome 15q11-13. Single nucleotide polymorphisms (SNPs), spaced on average by 13 kb were genotyped across the gene that had not been previously parsed in autism. Global significance for all marker p-values was obtained with region-wide empirical significance. Short range linkage disequilibrium was observed, but there is evidence for large amounts of recombination in the gene. A high LD haplotype of 4 SNPs was tested, including the two most significant markers, and was significant at $p = 0.0007$ when haplotypes of >2% were included and at $p = 0.006$ when haplotypes of >1% were included. Furthermore, we have genotyped other genes in the PWACR on this sample, namely GABRG3 and the maternally expressed genes UBE3A and ATP10C, using a dense array of SNPs across each gene. We are currently analysing these data and plan to present these plus gene-gene interaction analyses in the region.

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Testing and analysis of folate metabolism candidate genes for association with spina bifida meningomyelocele.

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Spina bifida meningomyelocele (SBMM) is the most common form of neural tube defect (NTD) that, with surgical and medical intervention, is compatible with survival. While, folate supplementation in the periconceptional period reduces both occurrence and recurrence risk of NTDs, the underlying mechanism is not understood. Thus, the folate metabolic pathway genes deserve study to determine their role in SBMM formation. We have tested single nucleotide polymorphisms (SNPs) across a number of the genes involved in folate metabolism in a sample including ~490 SB patients and their parents to assess for association. We utilized the SNPlex high throughout genotyping system to genotype approximately 1500 individuals. The SNPlex system allowed us to interrogate 47 SNPs in 14 genes of the folate metabolic pathway including: 1) MTHFR, MTRR, MTR, MTHFD1, BHMT, CBS, MAT2A, MAT2B, AHCY, DHFR involved in the enzymatic processes of folate metabolism and 2) RFC1, FOLR1, FOLR2, FOLR3, that respectively mediate transportation, delivery and secretion within this pathway. Thus far, we have successfully determined the genotype of 42 SNPs in ~1300 subjects. We are analyzing the data. The genotype data will allow us to determine the potential involvement of individual SNPs as well as variant haplotypes in these candidate genes that may confer risk for susceptibility to SBMM. It will also allow us to evaluate gene-gene effects by assessing two or more SNP combinations within different genes in the folate pathway. With this genotypic data in hand we can combine it with phenotypic data, such as brain dysmorphology and neurobehavioral outcomes, collected by one of our collaborating groups, to determine if single or combinations of multiple genetic variants can result in NTD susceptibility and the associated phenotypic variability observed in these disorders.

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Genome-wide association study of idiopathic Parkinson's disease using 27,000 microsatellite markers. *W. Satake[1, 2], Y. Hirota[1], Y. Momose[3], I. Mizuta[1], A. Oka[4], G. Tamiya[5], S. Sakoda[2], M. Yamamoto[6], N. Hattori[7], M. Murata[8], H. Inoko[4], T. Toda[1].* 1) Div. Clinical Genetics, Osaka Univ. Grad. Sch. Med., Osaka; 2) Dept. Neurol., Osaka Univ. Grad. Sch. Med., Osaka; 3) Dept. C.B.I., Grad. Sch. Med., Univ. Tokyo, Tokyo; 4) Dept. Mol. Life Sci. II, Tokai Univ. Sch. Med., Kanagawa; 5) Inst. Health Bioscience, Tokushima Univ. Grad. Sch. Med., Tokushima; 6) Dept. Neurol., Kagawa Pref. Central Hosp., Kagawa; 7) Dept. Neurol., Juntendo Univ. Sch. Med., Tokyo; 8) Dept. Neurol., NCNP, Tokyo, Japan.

Parkinsons disease (PD), one of the most common neurodegenerative diseases, is a complex disorder with multiple genetic and environmental factors influencing disease risk. To identify susceptibility genes for idiopathic PD, we performed a genome-wide association study with pooled DNA using approximately 27,000 microsatellite markers arranged at intervals of approximately 100 kb throughout the genome. For the 1st screening, we performed the association study with pooled DNA from 124 PD patients and 124 normal controls. We analyzed the PCR products with the GeneScan software, and compared the pattern of the PCR products of pooled DNA from PD patients with pooled DNA from controls by the PickPeak software, and found associations (p0.05, 2xm Fishers test) in approximately 8% (2,205 markers) of the markers. To exclude false positive associations, we performed 2nd and 3rd screenings using different sets of pooled DNA from 250 PD patients and 250 normal controls for each screening. 348 markers showed associations (p0.05, 2xm Fishers test) throughout all three screenings. We have been confirming these associations using individual samples of 624 PD patients and 624 normal controls. The markers which showed a consistent peak pattern throughout the three screenings were given priority for individual genotyping. So far, we have performed individual genotyping on 71 markers, and 16 markers still showed an association. Among these, 2 markers showed a stronger association (p0.001). Genes in linkage disequilibrium with these markers may be associated with the pathogenesis of PD.

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Frequency of mitochondrial DNA T8993G mutation in the ATPase 6 gene encoding subunit a, in patients with Alzheimer disease. *E. Martinez-Cano[1,2], M.P. Gallegos[3], M.A. Macias[4], G.G. Ortiz[1].* 1) Lab. Desarrollo-Envejecimiento, CIBO, IMSS, Guadalajara, Jalisco. Mexico; 2) Doctorado en Genetica humana, Universidad de Guadalajara; 3) Laboratorio de Genetica Molecular, CIBO, IMSS; 4) Departamento de Neurologia, HE, CMNO, IMSS, Guadalajara, Jalisco. Mexico.

BACKGROUND : Alzheimer disease (AD) is a progressive and neurodegenerative disorder characterized by cognitive and memory deterioration. Alzheimer's disease occurs gradually, and is not a normal part of the aging process, is the most common cause of dementia. Recently evidence indicates that a low in the energy generation of the mitochondria is characteristic that unifies the neurodegenerative diseases. In order to obtain direct evidence that mitochondrial function is altered in Alzheimer's disease. Different reports had pointed mutations in the mitochondrial ATPase gene. Mutation T8993G cause the NARP (neurogenic muscle weakness, ataxia and retinitis pigmentosa) and Leigh syndromes. OBJECTIVES: Determined the frequency of T8993G mutation of mitochondrial ATPasa6 gene, and their correlation with ATP synthase enzymatic activity, in AD patients and control group. MATERIAL AND METHODS: Controls (n=14) and the same number of patients with AD were selected. The AD patients were according to NINCDS/ARDRA criteria. The diagnosis was supported by Magnetic Resonance Imaging (MRI), Single Proton Emission Computed Tomography (SPECT) and the Mini Mental State Examination (MMSE) neuropsychological test. The ADN genomic extraction was according Miller method. Activity enzymatic was according Summer. RESULTS: The data revealed that the hydrolytic activity of ATP synthase was significantly increased in patients with AD (14.3 1.4 nmol PO4 min-1(mg protein)-1, n = 10) to compared with the control group (10.0 0.6 nmol PO4 min-1 (mg protein)-1, n = 10). The mutation T8993G was observed only in AD patients 71.42% (10/14) mutation homozygotes, 7.16% (1/14) heterozygotes and 21.42% (3/14) normal homozyotes. CONCLUSION: These results probably suggest that weak activity functional can be explained by T8993G mutation in ATP synthase that serve as proton channel for enzymatic activity.

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Exploring the association of glyceraldehyde-3-phosphate dehydrogenase (GAPD) gene and late-onset Alzheimer disease. *C.A. Browning[1], P.I. Lin[1], E.R. Martin[1], P.G. Bronson[1], G.S. Small[2], D.E. Schmechel[3], K.A. Welsh-Bohmer[3, 4], J.L. Haines[5], G.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Center for Human Genetics, Duke Univ, Durham, NC; 2) Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA; 3) Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC; 4) Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Duke University, Durham, NC; 5) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Previous linkage studies have shown that chromosome 12 harbors susceptibility genes for late-onset Alzheimer disease (LOAD). However, association studies of several candidate genes on this chromosome region have obtained conflicting results. Recently, Li et al. (2004) reported the association of the gliceraldehyde-3-phosphate dehydrogenase (GAPD) gene on chromosome 12p and the risk of LOAD. GAPD is closely related to neural apoptosis. Here, we carried out association studies on 12 single nucleotide polymorphisms (SNPs) in the GAPD gene and its paralogs. Overall we saw no evidence for single-locus association in the GAPD gene and its paralogs with LOAD, but there was marginally significant association in a SNP in the GAPD gene particularly in the later-onset LOAD subgroup (age-at-onset greater than the mean of 71 years). Family-based association tests showed that two SNPs in the GAPD gene and in PPM1H gene (a GAPD paralog) respectively were associated with the later-onset LOAD. None of these associations were significant after we performed multiple testing corrections. However, haplotype-based association analyses in the unrelated case-control sample revealed a two-locus haplotype significantly associated with the disease risk particularly for the later-onset LOAD (experiment-wide empirical p-value = 0.001). Additionally, we found suggestive evidence for the multi-locus effect of GAPD gene and PPM1H gene on the risk of LOAD, by using the Extended Multi-factor Dimensionality Reduction method. The results suggest that the role of susceptibility genes of minor effect, such as the GAPD gene for LOAD, may be revealed by multi-locus association analysis.

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A Meta-Analysis of the Relationship of the HOPA^{12bp} Polymorphism and Schizophrenia. *R. Philibert.* Dept Psychiatry, Univ Iowa, Iowa City, IA.

Uncommon polymorphisms, and in particular, balanced uncommon polymorphisms, present a significant challenge to our understanding of their role in behavior. Over a series of studies, we have demonstrated that an uncommon candidate gene polymorphism for schizophrenia, known HOPA^{12bp}, is the defining polymorphism for a large X-chromosome haplotype in population disequilibrium and that it is associated with a positive syndrome of psychosis. However, not all studies have shown this effect and our other studies have shown a broad, sex dependent effect of this allele on behavior and endocrine function. We reviewed prior studies and conducted meta-analysis of studies using probands of Northern European extraction. We find that the presence of the HOPA^{12bp} is a significant risk factor for psychosis for both men and women and suggest that differences in the case definition of schizophrenia may affect the strength of the association of this allele with behavioral illness.

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Pharmacogenetics of risperidone therapy in autism. *C. Correia[1], G. Oliveira[3], P. Santos[1], J. Almeida[3], A.M. Coutinho[1], C. Marques[3], C. Bento[3], A. Ataíde[3], T. Miguel[3], A.M. Vicente[1,2].* 1) Instituto Gulbenkian Ciencia, Portugal; 2) Instituto Nacional Saúde Dr Ricardo Jorge, Portugal; 3) Hospital Pediátrico Coimbra, Portugal.

Treatment of autism with the atypical antipsychotic risperidone is mainly effective in the control of disruptive behaviors associated with autism, while in some cases improving typical symptoms. The objective of this ongoing pharmacogenetic study is to identify genetic factors underlying the variability in individual response to this drug. Ten autistic patients (3 females, 7 males, mean age 12.73.6 years) were selected for risperidone therapy. Efficacy and tolerability was monitored at baseline and after one month, using the Autism Treatment Evaluation Checklist (ATEC) and assessing prolactin (PRL) levels and weight gain. Genotyping of *CYP2D6* alleles *3,*4,*5,*6 and gene duplication, and of polymorphisms in the *DRD2* and *HT2C* genes were performed. ATEC scores improved after one month in all patients except one, and were positively correlated with increase in PRL levels. Risperidone affinity for the D2 dopamine receptor is influenced by a 3UTR *DRD2* polymorphism, with allele A1 reported to be associated with PRL increase, and might therefore mediate the clinical response. Although the only patient with a PRL increase of clinical significance carried the A1 allele, we did not find an overall correlation between ATEC scores and *DRD2* genotypes, indicating an influence of other factors. The patient who did not respond to therapy had the *CYP2D6* gene duplication associated with the ultrametabolizer (UM) phenotype. Interestingly, this patient is obese but did not show any weight gain with treatment. Two other patients were homozygous for the poor metabolizer (PM) *CYP2D6*4* allele, but showed no evidence for increased side effects, while their ATEC scores improvement was variable. Overall, we found no correlation of ATEC scores, PRL levels or weight gain with *CYP2D6*, *DRD2* or *HT2C* genotypes. Our preliminary results indicate that the response to medication is determined by a complex interplay of factors, pointing out the need for a multiparametric approach to dissect genetic influences in drug response.

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Functional characterization of GRIK2 haplotypes associated with bipolar affective disorder. *S. Buervenich[1], R. Blumenthal[2], W.R. Corona[1], H.M. Alosh[1], R. Bachmann[2], J. Du[2], P. Yuan[2], N. Akula[1], H.K. Manji[2], S.D. Detera-Wadleigh[1], F.J. McMahon[1].* 1) Genetic Basis of Mood and Anxiety Disorders Unit, Mood and Anxiety Disorders Program, NIMH, Bethesda, MD, USA; 2) Laboratory of Molecular Pathophysiology, Mood and Anxiety Disorders Program, NIMH, Bethesda, MD, USA.

Bipolar affective disorder (BPAD) is one of the world's ten most disabling conditions with a suicide rate of up to 15 percent. Very little is known today about the biological causes of this disease, which has been shown very consistently in twin and adoption studies to be highly heritable. We recently identified association of GRIK2 (also known as GluR6) haplotypes with the disorder. The GRIK2 gene codes for a kainic acid-type ionotropic glutamate receptor expressed in several brain regions that have been implicated in the etiology of BPAD. Furthermore, it is involved in circadian-rhythm generating pathways and also has metabotropic effects that are conveyed at least in part by a PKC-dependent pathway, one of the pathways that have been shown to be regulated by the mood stabilizer lithium. We now report results from detailed investigation of the genomic sequence surrounding the markers defining the risk-haplotypes (rs6922753 and D6S1642). Re-sequencing of 24kb of genomic sequence revealed five new sequence changes, none of which, however, was located in coding regions. Functional studies using lymphoblast cell lines from homozygous risk-haplotype carriers support the hypothesis that the risk-haplotypes may be regulatory: Preliminary results from western-blot analyses on homozygous carriers of the rare allele of the SNP (located 7bp downstream of an exon/intron splice site) suggest high expression of a 55 kDa protein product (the wildtype GRIK2 product is approximately 115 kDa). Furthermore, expression of the shorter protein was found to be inducible by hydrocortisone treatment. Results from further analyses on the identity of the 55kDa protein product as well as studies on potential regulation of GRIK2 expression by mood stabilizers will be presented.

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Association of autism with serotonin-related genes. *J.D. Buxbaum[1, 2], N. Ramoz[1, 2], G. Cai[1, 2], J.G Reichert[1, 2], C.J. Smith[2], T.E. Corwin[2], J.M. Silverman[2], E. Hollander[2].* 1) Laboratory of molecular neuropsychiatry, Mount Sinai School of Medicine, New York, NY; 2) SEARVER center, Dept. of Psychiatry, Mount Sinai School of Medicine, New York, NY.

Autism (MIM#209850) is a complex neurodevelopmental disorder that is associated with hyperserotonemia in 30 percent of individuals, and functional variants of the serotonin transporter gene (SLC6A4) have been considered as susceptibility loci. To address the role of serotonin in autism, we have begun a detailed analysis of serotonin-related genes in autism. We have screened 10 variants of SLC6A4, including the insertion/deletion 5-HTTLPR in the promoter locus, 16 variants in TPH1 and TPH2, and 41 additional variants in 10 other serotonin-related genes, in 352 families with autism (90 simplex and 262 multiplex), including 653 autism subjects. Markers were analyzed for linkage disequilibrium using Haploview, linkage using the GENEHUNTER package, and association using family-based methods (Transmit, TDTPhase, and PDTphase). No evidence for linkage or association was found between autism and markers in SLC6A4 or TPH2. Some preliminary evidence for association was observed in two serotonin receptor genes, although this might reflect a Type I error. SLC6A4 and TPH2 may not be involved in the susceptibility to autism. To confirm association with autism and specific serotonin receptor genes, screening of additional SNPs in these genes and replication in additional populations are needed.

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Identification of a haplotype in *TAAR6* (*TRAR4*) associated with susceptibility to schizophrenia in a Caucasian population. *S.A. Paciga[1], Y.-T. Fan[2], K.M. Walton[3], D.P. King[1].* 1) Discovery Pharmacogenomics; 2) Non-clinical Biostatistics; 3) Discovery CNS; Pfizer Global Research & Development, Groton, CT 06340.

Schizophrenia is a chronic, often debilitating mental disorder with a substantial genetic risk that affects ~1% of the population worldwide. Estimates of the percentage of disease risk attributable to genetic variation exceed 80%. Several candidate genes have been described recently that show association with susceptibility to schizophrenia. Among these is a G-protein coupled receptor on chromosome 6q, *trace-amine-associated-receptor 6* (*TAAR6*, formerly *TRAR4*), which is expressed in multiple CNS tissues implicated in schizophrenia (Duan *et al*., AJHG 75:624-638, 2004). We describe here results of an independent genetic association study that provide additional positive evidence for the involvement of *TAAR6* in susceptibility to schizophrenia. We genotyped 11 single nucleotide polymorphisms spanning the *TAAR6* locus, including 9 markers reported by Duan *et al*., in 878 patients with schizophrenia or schizoaffective disorder (644 Caucasian, 234 African American) and 604 ethnically matched control subjects (407 Caucasian, 197 African American). DNA samples were genotyped using a 5 nuclease assay. In our populations of Caucasians and African Americans, no single-marker associations were found with the 11 SNPs tested. However, in Caucasians, a common two-marker haplotype (rs6907909, rs9373026) was identified that showed strong association with susceptibility to schizophrenia (p0.001). These data provide additional evidence supporting a possible role for *TAAR6* in schizophrenia.

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Evidence for autism loci in the chromosome 2q24-q33 region: association at AGC1, STK39, and ITGA4. *N. Ramoz[1, 2], J.G. Reichert[1, 2], C.J. Smith[2], T.E. Corwin[2], J.M. Silverman[2], J.D. Buxbaum[1, 2].* 1) Laboratory of molecular neuropsychiatry,Dept Psychiatry, Mount Sinai School of Medicine, New York, NY; 2) SEAVER center, Dept Psychiatry, Mount Sinai School of Medicine, New York, NY.

We recently reported linkage and association of AGC1 with autism (MIM#209850). Our current goal is to further assess the role of AGC1 in autism, and to explore whether there may be additional susceptibility loci in 2q24-q33. A total of 175 SNPs were genotyped in 352 families with autism (90 simplex and 262 multiplex), including 653 autism subjects. Eighty SNPs were selected to cover 40 Mb of the 2q24-q33 region. A further 95 SNPs within this region were chosen to increase the density of markers across 26 candidate genes, including 14 SNPs that encompass the AGC1 gene. Intermarker linkage disequilibrium was computed with Haploview. SNPs were analyzed for linkage using the GENEHUNTER (GH) program, and for association using family-based methods. Linkage analysis demonstrated a maximum multipoint non-parametric lod (NPL) score value of 1.9 (p=0.02) with SNPs across the AGC1 gene. The NPL value went up to 3 (p=0.0009) in a subset of the cohort with delayed onset of phrase speech. Two other peaks were detected that cover the STK39 gene and the ITGA4-NEUROD1 region. Significant association was observed between autism and SNPs in the AGC1, STK39, and ITGA4 genes. Evidence for haplotype association have been found. We have provided further evidence for linkage and association between autism and AGC1, and also potential linkage and association with other autism susceptibility loci in 2q24-q33.

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A-Synuclein gene SNCA variation is associated with Parkinsons disease. *A. Parsian[1], A.J. Parsian[1], B. Racette[2], J.H. Zhao[3], J.S. Perlmutter[2].* 1) Dept Pediatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Dept of Neurology, Washington University School of Medicine, St. Louis, MO;; 3) Dept of Epidemiology and Public Health, University College London, London, UK.

Several studies have replicated our preliminary report of an association between a dinucleotide repeat (SNCA) in a-Synuclein gene and sporadic Parkinsons disease (PD, Parsian et al., 1998). To replicate our previous finding in a larger sample and further determine the role of a-Synuclein in the development of PD, we screened a sample of 226 familial PD, 353 sporadic PD, and 130 controls with the SNCA marker and 3 SNPs (770, int3, and int4) in the gene. There was Hardy-Weinberg disequilibria only with SNP int3 that was excluded in the haplotype analysis. There was also significant difference in allele frequency between African American and American Indian groups for SNCA marker (p=0.03). These two samples were excluded from further analysis because of sample size. Comparison of allele frequency differences between PD and controls for single-locus was significant only for SNCA (0.0025). This result was further shown via haplotype analysis of SNCA-770-int4 markers. One of the interesting results of our haplotype analyses is with inclusion/ exclusion of SNP int3. The global case control association was not significant when the SNP int3 was included in four loci haplotype analysis. Otherwise, two and three loci haplotpes comparisons were significant. The explanation is that inclusion of locus in Hardy-Weinberg disequilibria in haplotype analysis will reduce power as shown by Epstein and Satten (2003). Our results indicate that SNCA locus may be in linkage disequilibrium with a mutation in the gene or itself could be a risk factor for PD.

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Parsing the genetic heterogeneity of chromosome 12q for Alzheimer disease by family-based association studies.

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Previous evidence has suggested that multiple genetic variants are involved in the etiology of late-onset Alzheimer disease (LOAD). So far no susceptibility genes for LOAD other than the apolipoprotein (APOE) gene have been conclusively identified due to inconsistent findings. We recently found increased evidence for linkage to a 12q region in autopsy-confirmed families particularly without linkage to alpha-T catenin gene on chromosome 10 (LOD score increased from 0.1 to 4.2; p 0.0001 for the increase in LOD score). Encouraged by these findings, we genotyped 83 single nucleotide polymorphisms (SNPs) in this 12q region spanning 6 megabases, and performed association analyses by using three programs: PDT, FBAT, and APL, for the overall sample (591 families) and an autopsy-confirmed subset (189 families). We observed no convergent significant results in the overall sample. However, in the autopsy-confirmed subset, we obtained convergent significant results at two SNPs: RS4759018 and RS1683151 in the activation transcription factor 7 (ATF7) gene. Additionally, we identified a multi-locus risk haplotype in the ATF7 gene (p = 0.0009) in this same subset. These two intronic SNPs are not in linkage disequilibrium with any functional polymorphism in the haplotype block retrieved from the HapMap data, but they are located in a highly conserved region. We further found that homozygotes for the C allele at RS1683151 had an increased risk for LOAD particularly for individuals carrying no APOE e4 allele. These results suggest that using subset and covariate analyses may help identify the elusive novel susceptibility genes for LOAD, such as ATF7 gene on 12q. Furthermore, the ATF7 gene may act independently of APOE gene to contribute to risk for LOAD. The ATF7 gene is related to the ATF/CREB family associated with neural plasticity and apoptosis, and hence may serve as a novel candidate susceptibility gene for LOAD.

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Locus-wide association study in the 5q33.1 region for schizophrenia susceptibility genes. *H. GOTO[1], H. SHIBATA[1], M. TAKAJI[1], H. NINOMIYA[2], N. TASHIRO[3], N. JARUSURAISIN[4], Y. FUKUMAKI[1].* 1) Medical Institute of Bioregulation, Kyushu University, FUKUOKA, FUKUOKA, Japan; 2) Fukuoka Prefectural Dazaifu Hospital Psychiatric Center, Dazaifu, FUKUOKA, Japan; 3) Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, FUKUOKA, FUKUOKA, Japan; 4) Department of Psychiatry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Schizophrenia is a common psychiatric disorder with a multifactorial background. As the Japanese Schizophrenia Sib-pair Linkage Group (JSSLG), we have reported that 11 chromosomal regions showed the nominal significance by genome-wide scan for linkage, although no evidence for significant linkage was obtained (JSSLG 2003). Out of these regions, we selected the 5q33.1 region for further analyses, where suggestive linkage with schizophrenia has been reported in multiple studies. We conducted locus-wide association studies of all annotated genes in the region (43 genes in about 4.9 Mb). We genotyped 100 Japanese cases and 100 controls for 131 single nucleotide polymorphism (SNP) makers distributed at intervals of less than 30 kb within each gene region. We observed significant associations of schizophrenia with 8 SNPs in 7 genes by single-point analyses and with 16 pairs of SNPs in 11 genes by haplotype analyses. All of the nominal significance was subsequently examined with additional 100 Japanese cases and 120 controls. We replicated the significant association of schizophrenia with one SNP in *SP329* by single-point analyses (P = 0.0434 in the first set and 0.0140 in the second set). We also replicated one significant haplotype association in *PERC* and *PDE6A* (P = 0.0031 in the first set and 0.0409 in the second set). We conclude that these genes were likely responsible for the genetic susceptibility to schizophrenia in the 5q33.1 region.

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Association of AKT1 with schizophrenia confirmed in the Irish Study of High Density Schizophrenia Families (ISHDSF). *D.L. Thiselton[1], V. Vladimirov[1], P-H. Kuo[1], B. Wormley[1], F.A. O'Neill[2], D. Walsh[3], K.S. Kendler[1], B. Riley[1].* 1) Dept. of Psychiatry, Virginia Commonwealth Univ, Richmond, VA; 2) Dept. of Psychiatry, The Queen's University, Belfast, Ireland; 3) The Health Research Board, Dublin, Ireland.

AKT1 was recently implicated as a schizophrenia susceptibility gene by association in a large family sample of European origin. Decreased AKT1 protein levels were detected in lymphocytes and brain tissue of schizophrenic patients. Two follow-up Japanese case-control studies provided conflicting replication evidence. AKT1 is critical to cell survival, growth, transcriptional regulation and nutrient metabolism via a signal transduction pathway involving PI3K and GSK3B. It appears to be involved in neurodevelopment and working memory formation, domains potentially impaired in schizophrenia. Functional studies suggest that defective PI3K-Akt signaling in schizophrenic brains may be due to decreased expression of dysbindin (DTNBP1). The presence of a high-risk DTNBP1 haplotype in the ISHDSF thus prompted our investigation of AKT1. Eight SNPs spanning AKT1, incorporating 4 from previous reports, were genotyped on the 270 ISHDSF families. The data was analysed for association with schizophrenia using FBAT, TRANSMIT, PDT and PDTphase, across 4 diagnostic categories. Although no single marker showed significant association with disease, the data was consistent with global association of the common alleles for haplotypes consisting of 3 SNPs or more, thus supporting association of AKT1 with schizophrenia. The 4-marker common haplotype for SNPs rs2498802-rs3803304-rs2494732-rs2498799 showed the most significant over-transmission using TRANSMIT (p=0.002), and a similar trend by FBAT and PDTphase. Analysing the data after stratification based on the DTNBP1 high-risk haplotype showed no evidence of epistasis. We are currently analyzing expression of AKT1 in schizophrenia brain RNA samples by real-time qPCR to see if the previously reported protein down-regulation is detected at the RNA level.

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Trace Amine Receptor 4 (TRAR4) gene is associated with Schizophrenia in the Irish Study of High Density Schizophrenia Families. *V.I. Vladimirov[1], D.L. Thiselton[1], P-H. Kuo[1], B. Wormley[1], J. Vittum[1], R. Ribble[1], F.A. ONeill[2], D. Walsh[3], K. Kendler[1], B. Riley[1].* 1) Dept. of Psychiatry, Virginia Commonwealth University, Richmond, VA; 2) Dept. of Psychiatry, The Queen's University, Belfast, Ireland; 3) The Health Research Board, Dublin, Ireland.

The Trace Amine Receptor 4 (TRAR4) gene was recently implicated as a potential liability locus for schizophrenia. The original study analyzed 192 schizophrenia pedigrees of European and African ancestry. Marker rs4305745 within TRAR4 was associated (P = 0.0014) after correction for multiple testing. We sought to replicate these findings in the Irish Study of High Density Schizophrenia Families (ISHDSF). We genotyped 15 single-nucleotide polymorphisms (SNP), including eight SNPs from the original study, in our 270 pedigrees. TRANSMIT analysis of single markers showed association with rs12189813 (P = 0.0084), which was confirmed by PDT. SNP markers rs8192622, rs8192624, rs8192625 SNPs lie in the coding sequence of TRAR4 and rs7772821 is in the 3 UTR 8bp downstream from the stop codon. Of those four SNPs, rs8192624 and rs7772821 show a trend towards association (P = 0.0676 and 0.0729 respectively), whereas the haplotype of these 4 markers was significantly associated with the disease (P = 0.006). Pedigree disequilibrium testing (PDT) of single markers showed association only with a one SNP, rs12189813 (p = 0.0057), which is 35kb away from the 3 end of TRAR4. However, a haplotype of four SNPs (rs7772821, rs4305745, rs6903874, rs7765655) which extend from within the 3 UTR of the gene showed significant association with the disease (p = 0.0000529). Although different analytic methods emphasize different subsets of the markers tested, the overall evidence for association of this gene in our sample is highly significant.

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Haplotype analyses of the serotonin receptor 2C gene (HTR2C) show evidence of association with male affected with autism spectrum disorders. *G.M. Orabona[1], E.S Moreira[1], L.O. Avelar[1], K. Griesi-Oliveira[1], E. Vadasz[2], L.M. Biason[2], S. Matiolli[1], P.A. Otto[1], M.R. Passos-Bueno[1].* 1) Centro de Estudos do Genoma Humano/Departamento de Biologia/IB, Universidade de São PauloInstituto de Biologia, USP, São Paulo, SP., Brazil; 2) SEPIA, Instituto de Psiquiatria/FM, Universidade de São Paulo, São Paulo, Brazil.

Autism spectrum disorder (ASD) is a group of behaviorally defined neurodevelopmental disabilities with core deficits in socialization and communication, and restricted and repetitive patterns of interests or behaviors. Fewer is known about its genetic mechanisms but there are evidences showing that it involves multiple biochemical pathways. Serotonin has long been implicated in the development of autism since at least 30% of subjects are hyperserotonemic. A recent mathematical model related the serotonin receptors with mechanisms leading to abnormalities in the regulation of serotonergic system in ASD. In our study, we investigated the serotonin receptor 2C gene (HTR2C) for association with ASD. The HTR2C is widely expressed in brain regions implicated in behaviors altered in ASD such as amygdala and hippocampus. Our sample consists of 163 subjects from Brazil (132 males: 31 females), diagnosed using DSM-IV and ADI-R methods and previously excluded for known medical and genetic conditions associated with ASD. The control group consisted of 274 individuals (164 males: 110 females) with comparable ancestry with our subjects. Four SNPs were genotyped (SNuPE) and analyzed for case-control haplotype analysis: two were intronic SNPs rs2248440, rs5946005, one functional rs6318 (Cys23Ser) and a non-described polymorphism 25bp next to the intron encoded snoRNA HBI-36 gene (IVS2 + 16895 G A), all in H-W equilibrium. A total of eight haplotypes were observed both in cases and controls with different levels of linkage disequilibrium. We observed that one of them is significantly associated with male subjects (P=0,0008 - broader diagnosis of ASD; P=0,001 - narrow diagnostic of autism). This is the first report of association of HTR2C and ASD, further highlighting the importance of the serotonin system with this disorder.

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Association between iNOS and early-onset Parkinson disease. *D.B. Hancock[1,2], B.S. Wheeler[2], E. Tegnell[1], M.A. Hauser[1], E.R. Martin[1], J.M. Vance[1], W.K. Scott[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) University Program in Genetics and Genomics, Duke University, Durham, NC.

Parkinson disease (PD), the second most common neurodegenerative disorder following Alzheimer disease, is characterized by progressive depletion of dopaminergic neurons within the substantia nigra. Excess synthesis of nitric oxide (NO), a messenger molecule with diverse physiological functions, has been implicated as a neurotoxic effector in PD by inducing numerous detrimental cellular effects including reduced mitochondrial complex I activity. Previous case-control studies observed an association between sporadic PD risk and the main source of NO synthesis, iNOS (inducible nitric oxide synthase), and suggested participation by iNOS as a modifier in PD. In a genomic screen to identify genes influencing age-at-onset of PD, we previously reported linkage (LOD=1.93 at 25cM) to chromosome 17 in the vicinity of the iNOS location (51cM). Consequently, we set out to investigate a possible iNOS association with PD in the context of age-at-onset. We genotyped seven coding and intronic single nucleotide polymorphisms (SNPs), including the previously implicated SNP rs1060826, in 291 multiplex (two or more members with PD) and 483 singleton families. Allelic association analysis using the Association in the Presence of Linkage (APL) test revealed significant association between rs1060826 and PD risk in the 78 families with at least one member affected with PD prior to age 40 (p=0.001). The association remained significant in the 290 families with onset before age 50 but was somewhat less significant (p=0.005). Quantitative Transmission Disequilibrium Test (QTDT) analysis using age-at-onset as the trait demonstrated a significant association of rs1060826 with age-at-onset in the 291 multiplex and 483 singleton families (p=0.006). These data support the prior association of the iNOS SNP rs1060826 with PD but limit the effect to earlier onset of PD. As rs1060826 is a synonymous change with no known functional significance, these data suggest that the true functional variant associated with PD likely resides in linkage disequilibrium with rs1060826 and remains to be identified.

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Multiple candidate gene analysis identifies -*synuclein* as a susceptibility gene for sporadic Parkinsons disease. *I. Mizuta[1], W. Satake[1], A. Oka[2], H. Inoko[2], M. Yamamoto[3], N. Hattori[4], M. Murata[5], T. Toda[1,6].* 1) Div Clinical Genetics, Depat Medical Genetics, Osaka Univ Grad Sch Med, Suita, Osaka, Japan; 2) Dept Mol Life Sci, Tokai Univ Sch Med, Kanagawa, Japan; 3) Dept Neurol, Kagawa Prefectural Central Hosp, Kagawa, Japan; 4) Dept Neurol, Juntendo Univ Sch Med, Tokyo, Japan; 5) Dept Neurol, Musashi Hosp, NCNP, Tokyo, Japan; 6) CREST, JST, Saitama, Japan.

Parkinsons disease (PD), one of the most common human neurodegenerative diseases, is characterized by loss of dopaminergic neurons in the substantia nigra of the midbrain. PD is a complex disorder, with multiple genetic and environmental factors influencing disease risk. To identify causative genes for sporadic PD, we performed case-control association studies of 268 single nucleotide polymorphisms (SNPs) in 121 candidate genes. In two independent case-control populations, we found that a SNP in -*synuclein (SNCA)*, rs7684318, showed the strongest association with PD (P=5.0x10^{-10}). Linkage disequilibrium (LD) analysis using 29 SNPs in a region around rs7684318 revealed that the entire *SNCA* gene lies within a single LD block (D0.9) spanning about 120 kb. A tight LD group ($r^2$0.85) of six SNPs, including rs7684318, associated most strongly with PD (P=2.0x10^{-9}-1.7x10^{-11}). Haplotype association analysis did not show lower *P*-values than any single SNP within this group. Allele C of rs7684318 was positively associated with PD, more strongly in the recessive model than in the dominant model. *SNCA* expression levels tended to be lower for the CC genotype of rs7684318 than for CT and TT in lymphoblastoid cells and autopsied brain tissue. These findings establish *SNCA* as a definite susceptibility gene for sporadic PD and suggest that reduced SNCA expression also relates to the etiology of the disease.

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Mutation Analysis of the Rett syndrome gene (MECP2) exon 1 in autism and mental retardation, and genetic studies of the distal portion of chromosome Xq. *B. Stachowiak[1,2], S.D. Menon[3], C. Harvey[1,2], A.K. Mensah[1,2], G.N. Mnatzakanian[4], S.E. Alfred[1,2], R. Guo[4], S.W. Scherer[4], B.A. Minassian[4], A.K. Srivistava[3], J.B. Vincent[1,2].* 1) Molecular Neuropsychiatry & Development Lab, Ctr Addiction & Mental, Toronto, ON, Canada; 2) Neurogenetics Section, CAMH and Dept. of Psychiatry University of Toronto; 3) J.C.Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood,; 4) . Program in Genetics and Genomic Biology, and 5. Autism Research Unit, The Hospital for Sick Children, Toronto.

A new isoform for the Rett syndrome gene, MECP2, was recently identified, that includes coding sequence from exon 1, and is the predominant transcript in the central nervous system. This sequence encodes polyalanine and polyglycine stretches within the N-terminal portion of Mecp2, and may confer novel properties to the protein. We screened autism, mental retardation (MR), and control populations for sequence variation within this region, and identified variation in ~0.6% of MR cases screened (N=1,274). Most of these variants occur within a trinucleotide repeat region and result in change in number of alanine or glycine residues within the repeat stretches. We suggest some of these variants may be a relatively frequent cause of non-specific mental retardation or developmental delay. Skewed X-inactivation is frequently associated with X-linked mental retardation. Thus, we have performed X-inactivation studies by quantitative genotyping of a microsatellite marker at the androgen receptor gene. We have also attempted to confirm evidence of genetic linkage for autism on the distal portion of chromosome Xq, by genotyping 9 microsatellite markers across the region of interest.

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Novel splice isoforms for NLGN3 and NLGN4 with possible implications in autism. *Z. Talebizadeh[1], D.Y. Lam[1], M.F. Theodoro[1], D.C. Bittel[1], G.H. Lushington[2], M.G. Butler[1].* 1) Childrens Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) University of Kansas, Lawrence, KS.

Mutations in two X-linked neuroligin genes, NLGN3 and NLGN4, have been reported recently in individuals with autism spectrum disorders (ASD). To further characterize the role of these genes in ASD, we screened lymphoblastoid cDNA from 10 young autistic females with X chromosome inactivation skewness (80:20%) and 30 non-autistic males and females. Interestingly, a novel NLGN4 isoform lacking exon 4 was identified in one of the autistic females which was predicted to result in an in-frame exclusion of 62 amino acids containing a highly conserved esterase-like domain critical for the function of neuroligins as postsynaptic adhesion molecules. Monoallelic expression of NLGN4 was also seen in this subject as well as 11 of 14 informative females (both autistic and non-autistic) using a SNP found at 3 UTR. This informative SNP indicated that skipping of exon 4 occurred *de novo* on the paternal allele. In addition, the NLGN3 transcript was present in two isoforms (with and without exon 7) in 9 of 10 autistic females, 30 non-autistic subjects including parents of the autistic female not producing a transcript without exon 7 and a control female brain. The isoform lacking exon 7 absent in our autistic female has not previously been described and was predicted to produce a truncated product with premature termination following exon 6. We suggest the novel truncated NLGN3 product may have a regulatory role as reported for other proteins (e.g., vasopressin receptor) by attenuating the function of the full length isoform resulting in a reduction of the mature NLGN3 protein. To evaluate the impact of these two isoforms on protein structure, 3D structures of the NLGN3 and NLGN4 proteins were characterized using comparative modeling (e.g., Modeller program) and significant changes were suggested in the protein cores. Our study further suggests that genetic alterations besides mutations (e.g., splice variants) may lead to potentially abnormal function of neuroligins in the causation of ASD.

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Mutational analysis of EFHC1 gene in families with JME. *F. Annesi[1], F.E. Rocca[1], A. Labate[1], P. Tarantino[1], S. Carrideo[1], I.C. Cirò Candiano[1], D. Civitelli[1], P. Spadafora[1], E.V. De Marco[1], A. Gambardella[1,2], G. Annesi[1].* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Juvenile myoclonic epilepsy (JME) is a common form of generalized epilepsy that starts in adolescence. Recently, a major JME locus was mapped to chromosomal region 6p12.p11 and it was associated with mutations in the EFHC1 gene. This gene contains 11 exons and encodes a protein of 640-amino acids that contains 3 DM10 domains and an EF hand calcium-binding motif. In this study, we screened for mutations in the EFHC1 gene ten families from southern Italy in which at least two members had a typical form of JME. Ten families were selected and families with fewer than two affected members were excluded from the study. In each patient the diagnosis of JME was done after a detailed clinical and EEG investigation, according to the ILAE criteria. All investigated subjects signed informed consent to the genetic investigation. DNA was isolated from peripheral blood lymphocytes by standard methods. PCR amplification for each exon of EFHC1 gene was performed using intronic primers. Sequencing of PCR products were determined on the ABI 3100 automated sequencer using ABI PRISM sequencing kit according to the manufacturers protocol. At least the proband of each family with JME phenotype was screened for mutational analysis of EFHC1 gene. We have identified a previously described heterozygous dominant (F229L) mutation in one family. Recently, EFHC1 gene has been associated with JME in six out 44 families from Belize, Los Angeles, and Mexico. The authors detected 3 heterozygous mutations (F229L, D210L, D253Y) and one double heterozygous mutation (P77T, R221H) that cosegregated with epilepsy among 21 affected members of 6 families. Several unaffected members had mutations, indicating low disease penetrance. The results of our study are important as they extend for the first time the distribution of EFHC1 mutations to Caucasian populations. Moreover, our data provide further evidence for the high level of genetic heterogeneity associated with JME, as most of our JME families did not carry any mutations.

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A High Throughput Mutation Screen of Human Ion Channel Genes in Episodic Neurological Disorders. *R. Lafreniere[1], M. Simoneau[1], J.-F. Poulin[1], N. Gupta[1], F. Lafreniere[1], K. Boisvert[1], M. Albanese[1], M.K. Charles[1], S. Laurent[1], G. Ebers[2], Z. Cader[3], J. Sequeiros[4], J.M. Pereira Monteiro[5], G. Turecki[6], M. Alda[6], P. Grof[7], S. Chouinard[8], B. Brais[8], P. Cossette[8], G.A. Rouleau[1,8].* 1) Emerillon Therapeutics Inc., Montreal, Canada; 2) Wellcome Trust Centre for Human Genetics, Oxford, UK; 3) Oxford University, Oxford, UK; 4) UNIgene, IBMC, University of Porto, Portugal; 5) Hospital Santo António, Porto, Portugal; 6) McGill University, Montreal, Canada; 7) University of Ottawa, Ottawa, Canada; 8) Universite de Montreal, Montreal, Canada.

Common neurological conditions such as epilepsy and migraine are episodic disorders showing significant heritability. Mutations in several ion channel genes have been identified as the genetic basis of these disorders in some patients. Additional diseases such as bipolar disorder, essential tremor and Tourette syndrome share some aspects of episodic disorders, and may be caused by dysfunction of ion channel genes. In an effort to identify relevant drug targets for these neurological disorders, we have initiated a high throughput mutation detection screen of brain-expressed human ion channel genes. Our goal is to screen 150 genes in 368 DNA samples from unrelated patients with these five disorders. Patient samples were selected based on clear family history and diagnosis, and availability of additional family samples to allow segregation analysis. Samples are screened for DNA variants using denaturing HPLC and DNA sequencing. RFLP analysis or allele-specific oligonucleotide hybridization is then used to determine allele frequencies. To date, we have screened 276 unrelated patient samples through 100 ion channel genes, identifying a total of 659 variants. Of these, 96 missense mutations are being further validated using: (1) association studies comparing allele frequencies in patients and controls; and (2) segregation analysis in extended pedigrees. Several interesting candidate genes have been identified and will be presented. Identification of genetic factors causing these neurological disorders will benefit patients and families through better molecular diagnosis and improved therapeutics.

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Autosomal Dominant Inheritance of Stuttering in a West African Population. *B. Levis[1, 2], J. Lukong[3], J. Mundorff[4], D. Drayna[1, 2].* 1) NIDCD, National Institutes of Health, Rockville, MD, USA; 2) Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD, USA; 3) Speak Clear Association of Cameroon, Douala, Republic of Cameroon, Africa; 4) Hollins Communications Research Institute, Roanoke, VA, USA.

Stuttering is a common speech disorder characterized by interruptions in the flow of speech, including prolongations of sounds, repetition of sounds or syllables and/or silent blockages. Although the fundamental causes of stuttering are unknown, both genetic and non-genetic factors are involved. Evidence for genetic factors in stuttering come primarily from twin studies, suggesting concordance rates of ~60% and ~20% in MZ and DZ twins, respectively, and previously published linkage studies of stuttering identifying loci on chromosomes 12 and 18. In our further investigations of the genetic causes of stuttering, we have identified a cluster of families in which this disorder occurs as a trait with a simple inheritance pattern. We have ascertained 5 families from North West Province in the Republic of Cameroon that present with familial nonsyndromic stuttering that occurs at a frequency of ~0.40 (0.35 - 0.44; s.d. = 0.039). These families contain 3-5 generations, and persistent adult stuttering has been documented in up to 3 generations using standardized recorded speech samples. These families contain a total of 278 affected individuals, ranging from 15 to 106 per family. Affected individuals in these families show no neurological or developmental deficits other than stuttering. Although English is spoken in these families, all five families also speak Bamileke, one of the ~270 languages of Cameroon as their native tongue. All five families are descendants of Bantu lineage, and originate in a limited geographic region of Cameroon near the border with Nigeria, in which a high prevalence of stuttering has been reported. These features are consistent with the view that a common founder mutation may underlie stuttering in these families, and provides a unique and powerful population to help identify genetic effects in developmental stuttering.

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The sortilin-related receptor SORLA is functionally and genetically associated with Alzheimer Disease. *Y. Meng[1], E. Rogaeva[2], J.H. Lee[3], Y.J. Gu[2], T. Kawarai[2], T. Katayama[2], P. Erlich[1], C.T. Baldwin[1], R. Cheng[3], H. Hasegawa[2], F. Chen[2], N. Shibata[2], K.L. Lunetta[1], L.A. Cupples[1], Y. Song[4], P.E. Fraser[2], D. Westaway[2], R. Mayeux[3], L.A. Farrer[1], P. St George-Hyslop[2].* 1) Boston University, Boston, MA; 2) University of Toronto, Toronto, Canada; 3) Columbia University, New York, NY; 4) University of Hong Kong, Hong Kong.

Several lines of evidence implicate the retromer complex, which mediates subcellular protein sorting, in processing the amyloid precursor protein (APP). To test the hypothesis that genes in the retromer trafficking pathway modulate risk for Alzheimer disease (AD), we investigated associations between AD and 3 SNPs located in each of the coding sequences for the SORT1 (1p21-p13), SORCS2 (4p16), VPS26 (10q21), SORCS1 (10q23-q25), SORCS3 (10q23-q25), SORLA (11q23-q24), and VPS35 (16q12) genes in 7 large, family-based and case-control AD cohorts of Caucasian, African American and Hispanic heritage. Based on evidence of association with SORLA in more than one dataset, we genotyped 29 SNPs spanning ~175 kb in the SORLA region. Significant association (0.0007 p 0.05) was observed with 23 SNPs in at least one dataset. Adjustment for APOE genotype, age and sex had little impact on the inferences about association in any of the datasets. Haplotype analyses using a sliding window of 2-6 contiguous SNPs confirmed an association between SORLA and AD. The greatest consistency of results across datasets was observed in one region encompassing 3 SNPs, however, the most significant results were observed in one dataset with overlapping 6-marker haplotypes in another region spanning 16 SNPs (global p-values 0.001). These results support the existence of multiple unique pathogenic variants, each of which might be associated with AD in different populations. Functional studies investigating the effects of increasing or decreasing the expression of SORLA on the secretion of A showed that suppression of the expression of SORLA in vitro increases the production of A. Our genetic and functional data suggest that variants in SORLA increase AD risk, and this increased risk might arise from changes in APP processing.

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Genetic Association between endothelial Nitric Oxide Synthase (NOS3) and Alzheimer disease: The MIRAGE Study. *A. Akomolafe[1], K.L. Lunetta[2], P.M. Erlich[3], L.A. Cupples[2], C.T. Baldwin[3], M. Huyck[3], R.C. Green[2,3], L.A. Farrer[2,3].* 1) Morehouse School of Medicine; 2) Boston University Schools of Public Health and; 3) Medicine.

Evidence suggests that vascular and inflammatory components may be important in the etiology of Alzheimer disease (AD). Studies in UK and Italian samples have reported significant associations between the Glu allele of the Glu298Asp polymorphism in NOS3 and late-onset AD, while studies in two other Italian samples, as well as Japanese, US white and Hispanic samples failed to find association. These studies did not consider Glu298Asp in the context of other NOS3 polymorphisms. We genotyped Glu298Asp and 10 additional SNPs spanning 25.3kb of NOS3 in the MIRAGE Study sample, collected from multiple centers in the US, Canada and Germany. We used a generalized estimating equation approach to test for association in African American (N=235) and Caucasian (N=259) subgroups of AD affected patients, their unaffected siblings (N=144, N=326), and unrelated spouse/neighborhood controls (N=82, N=127). The Glu298 allele was more common in the African American than the Caucasian sample (allele frequency 0.91 vs. 0.65). The Glu298 allele was associated with higher risk of AD in the African American (p=0.038) but not the Caucasian subgroup. We tested sliding windows of 2-5 SNP haplotypes along the gene for association with AD in the unrelated case-control samples. In the African Americans, haplotypes including the Glu298Asp SNP and an intron boundary SNP 2.8kb downstream were the most significantly associated with AD risk (global p=0.008). In the Caucasians, low frequency haplotypes in the 3 end of the gene were protective (global p=0.0002); no haplotypes were significantly associated with AD risk. Adjusting for APOE-4 carrier status did not substantively affect our results. This study is the first to examine the association of NOS3 Glu298Asp in an African American AD sample, and the first to look at multiple NOS3 polymorphisms in the context of AD risk. Our results give further evidence that the Glu298-AD association is sample-dependent, and suggest that other variants within NOS3 may also influence AD risk.

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A scheme for comprehensive analysis of candidate gene associations: TNF and schizophrenia genesis. *B. Shirts[1], M. Bamne[1], J. Kim[2], M. Talkowski[1], J. Woods[1], R. Yolken[3], V. Nimgaonkar[1].* 1) Dept Psychiatry & Human Gen, U of Pittsburgh, Pittsburgh, PA; 2) Dept of Psychiatry, Catholic U of Korea, Seoul, Korea; 3) Stanley Division of Neurovirology, Johns Hopkins, Baltimore, MD.

Several association studies and a recent meta-analysis have indicated that the TNF -308G>A polymorphism may contribute to schizophrenia (SZ) risk in Caucasian populations. We designed a study to address the following explanations for differing findings in past association studies: 1) Rare risk polymorphisms in LD with the associated SNP may be present; 2) Exposure to infectious agents may cause increased risk in combination with associated SNPs; 3) Differential TNF expression may affect risk and be caused by one or several common haplotypes. We used a systematic approach to address these issues. We sequenced the entire TNF gene including introns and 3kb of 5 and 3 UTRs in DNA pooled from 115 Caucasian SZ cases and 200 controls to identify over-represented polymorphisms in SZ cases. We then selected 8 SNPs, which we genotyped individually in 250 cases and 250 controls. Using this information we identified 6 common haplotypes (freq > 1%) in our Caucasian sample. We analyzed genotype and haplotype associations and evaluated subgroups defined by gender, HSV1, HSV2, CMV, and Toxoplasma exposure. Finally, we used a dual-luciferase expression assay to quantify TNF expression driven by each common promoter haplotype. We identified 18 SNPs with frequencies > 5% in SZ cases, none of which were novel. Neither individual SNPs nor haplotypes were associated with SZ risk in our sample or in subgroups of SZ cases defined by infectious disease exposure or gender. Differences in TNF promoter driven expression were not correlated with case/control haplotype frequency differences. Our study shows no evidence to implicate TNF in SZ risk. We cannot rule-out the possibility of other genes in LD with TNF being associated with SZ. We have addressed the possibility of over-represented polymorphisms by pooled DNA sequencing, stratified for infectious exposure, and addressed the possibility of differential expression. We suggest these methods are generalizable and will be useful in association studies of other genes.

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Genetic and epigenetic analysis of MeCP2 expression defects in autism and other neurodevelopmental disorder brain samples. *R.P. Nagarajan, A.R. Hogart, Y. Gwye, J.M. LaSalle.* Dept of Med Micro & Immun, UC Davis, Davis, CA.

Mutations in the gene *MECP2*, encoding methyl CpG binding protein 2 (MeCP2), are responsible for most cases of Rett syndrome (RTT), an X-linked neurodevelopmental disorder. Both RTT and autistic disorder share a loss of social, cognitive and language skills and a gain in repetitive stereotyped behavior, following apparently normal prenatal and perinatal development. Although *MECP2* coding mutations are a rare cause of autism, MeCP2 expression defects were previously found in autism brain. To determine the frequency of MeCP2 expression defects, MeCP2 protein expression in autism and other neurodevelopmental disorders was compared with control postmortem cerebral cortex samples on a large tissue microarray. MeCP2 isoform-specific immunofluorescence was detected with laser scanning cytometry, normalized to control histone H1 staining and confirmed by Western blotting. Significantly reduced expression of total MeCP2 was found in 11/14 autism brain samples compared to multiple age-matched controls, whereas 9/14 showed significant MeCP2_e2 expression defects. In addition, significantly reduced MeCP2 protein expression was observed in other autism spectrum disorders, including Angelman syndrome (4/4), Prader-Willi syndrome (3/4), and Down syndrome (3/5). To investigate the possibility that some MeCP2 expression defects in autism are caused by mutations in non-coding regulatory regions of *MECP2*, DNA sequencing was performed. While 4/5 autism DNA samples showed wild-type sequence (-1700 to +300), one autistic female with significantly reduced MeCP2 expression was heterozygous for a novel T>C transition. This variant was not found in 80 autistic individuals and 15 controls, suggesting a rare regulatory mutation. As an alternative explanation for reduced MeCP2 expression, methyl-sensitive restriction digestion and bisulfite sequencing are being performed to detect putative aberrant *MECP2* promoter methylation in male autism DNA samples. These results demonstrate that MeCP2 expression defects are a common, but not unique, characteristic of the autism brain and suggest that both genetic and epigenetic factors play a role in abnormal expression of MeCP2 in autism.

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Screening for PINK1 mutations in patients with early- and late-onset Parkinson's disese. *I.C. Cirò Candiano, F.E. Rocca, P. Tarantino, F. Annesi, S. Carrideo, P. Spadafora, D. Civitelli, E.V. De Marco, G. Annesi.* 1Institute of Neurological Sciences, National Research Council, Mangone (CS), Italy.

Objective Mutations in the PTEN-induced kinase (PINK1) gene located within the PARK6 locus on chromosome 1p35-p36 were recently identified in patients with recessive early-onset Parkinsons disease. In this study, we performed a mutational analysis of PINK1 in 12 families, 4 with early-onset and 8 with late-onset parkinsonism, and in 23 sporadic patients with early-onset (age of onset 45 years of age) Parkinsons disease. The patients originated from Southern Italy. Mutations in parkin and DJ-1 were excluded previously in all patients. Materials and methods DNA was extracted from peripheral blood according to standard protocols. The 8 exons of the gene with their exon-intron boundaries were amplified using polymerase chain reaction (PCR). Obtained fragments were sequenced on ABI3100 automated DNA sequencer (Perkin Elmer- Applied Biosystems). Results A novel homozygous deletion (889delG) was detected in the affected members of a consanguineous family with early-onset parkinsonism and was absent in 100 normal chromosomes. We also identified several exonic and intronic polymorphic variants, most of whom already described. Discussion and conclusions Sequence analysis of the PINK1 gene led to the identification of a homozygous deletion in exon 4 in two sibs from a consanguineous family with early-onset parkinsonism. The deletion produces a premature stop codon and a protein lacking in most of the kinase catalytic domain. The screening of the remaining patients revealed presence of several polymorphic variants. These findings confirm that recessive mutations in PINK1 cause early-onset parkinsonism, although PARK6 is not a common locus for PD in our population.

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Interaction or coincidence: mutations in *PARKIN* and *LRRK2*. *I.F. Mata[1,2], C. Huerta[2], C. Lahoz[3], M. Blazquez[3], L.M. Guisasola[3], C. Salvador[3], R. Ribacoba[4], C. Martinez[5], M.J. Farrer[1], V. Alvarez[2].* 1) Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Laboratorio de Genetica Molecular, Instituto de Investigacion Nefrologica (IRSIN-FRIAT), Hospital Universitario Central de Asturias, Oviedo, Spain; 3) Servicio de Neurologia, Hospital Universitario Central de Asturias, Oviedo, Spain; 4) Servicio de Neurología, Hospital Alvarez-Buylla, Mieres, Spain; 5) Servicio de Neurología, Hospital de Cabuenes, Gijon, Spain.

The genetic contribution in Parkinsons disease (PD) has been established over the last decade. Mutations in seven genes (*SNCA, PRKN, UCHL-1, PINK1, DJ1, MAPT* and *LRRK2*) have been associated with the development of parkinsonism. Pathogenic substitutions in parkin are the most frequent genetic cause of PD, and an extensive list of mutations has been identified worldwide. However, mutations in the recently cloned *LRRK2* gene have been shown to explain a substantial number of late-onset typical PD cases. The G2019S pathogenic substitution has been reported to account for up to 41% of the familial cases in North Africa although this frequency decreases to less than 5% in European populations. An R1441G mutation is also frequent (~3%) in PD patients from Northern Spain. The objective of this study was to determine the frequency of mutations in the PRKN gene and the presence or absence of three *LRRK2* mutations. We screened more than 300 PD patients, from Asturias in the North of Spain, for variants in the Parkin gene using Single Strand Conformation Analysis (SSCA). All those samples showing a different electrophoresis pattern were sequenced and mutations were confirmed by restriction enzyme digestion. R1441G, R1514Q and G2019S ABI assay-by-design probes were used to identify these substitutions. We identified 27 patients carrying 10 different parkin pathogenic substitutions; the N52/Stop80 and M192V are the most frequent in our population. Two of the patients were compound heterozygous carrying two and three parkin mutations each. Interestingly, four of our heterozygous parkin carriers also carried one of the three Lrrk2 substitutions analyzed. The impact of carrying both a *PRKN* and *LRRK2* mutation must now be examined at the clinical and pathological level.

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Follow-up of the 7q32 and 16p13 regions linked to epilepsy-related photosensitive families. *D. Pinto, D. Kasteleijn-Nolst Trenite, B. Westland, D. Lindhout, B. Koeleman.* DBG-Department Medical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands.

Recently, we mapped two susceptibility loci for photosensitivity (PPR) at 7q32 and 16p13 in 16 PPR-multiplex families with prominent myoclonic epilepsy background. Here, we present the follow-up studies performed on these two regions. The inheritance model for these two loci was explored, showing that the 16p13 locus may have a recessive inheritance. A two-locus linkage analysis was also performed to test for different interaction models. Several potential candidate genes reside in these regions. The genes encoding for the metabotropic glutamate receptor 8 (GRM8), the cholinergic-muscarinic type 2 acetylcholine receptor (CHRM2), the voltage-dependent T-type calcium channel alpha-1H subunit (CACNA1H) and the somatostatin receptor type 5 (SSTR5) are known to participate in the neuromodulation of cortical dynamics. To evaluate the relevance of these high-ranking positional and functional candidate genes in the susceptibility for PPR in our families, we screened two patients from each family and Dutch controls for mutations in the coding region, 100 bp of exon-intron boundaries, 5 and 3untranslated regions and promoter regions. We found novel variants not reported in the NCBI dbSNP, and detected several known variants which were also present in Dutch controls. We are currently evaluating their familial segregation and putative biological meaning. The variants identified may be useful to conduct future association studies. The identification of susceptibility gene for PPR would advance our understanding of epileptogenesis.

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Clinical follow-up of multiplex late-onset Alzheimer Disease families. *R.M. Carney, P.C. Gaskell, W.K. Scott, M.A. Slifer, C.M. Hulette, K.A. Welsh-Bohmer, D.E. Schmechel, J.M. Vance, M.A. Pericak-Vance.* Duke University Medical Center, Durham, NC.

Eighty-one families with multiple living members affected with AD were ascertained for genetic analyses of late-onset Alzheimer Disease (LOAD; mean age-at-onset 60 years). Initial data collection included pedigree and clinical information establishing affection status and a blood sample. Two to 17 years after original contact, we successfully re-contacted 63 LOAD families to update our clinical and family history information. Follow-up data include clinical status changes, age at onset, and supplemental pedigree information.

Of those 230 individuals who were previously unclear or unaffected, 48 converted to affected (10/19 [53%] of those initially unclear and 38/211 [18%] of those initially unaffected) upon clinical re-evaluation. Additionally, 25 of the 211 (12%) of the initially unaffected converted to unclear. Expanding the family history data to include information on new family members identified an additional 20 affected individuals.

We were able to augment clinical diagnosis with neuropathological diagnosis on 101 individuals through our autopsy program. Despite all 101 individuals presenting with a clinical diagnosis of AD, only 73 individuals had a pathologic diagnosis of AD alone; 9 had non-AD dementia, and 19 had AD and additional neuropathologic features.

The variation in neuropathologic diagnoses despite a uniform clinical diagnosis further obfuscates the dissection of this complex disorder. Gene X gene or gene X environment interactions may alter neuropathologic features, similar to the range of pathologic findings seen in individuals with Parkinsonism carrying identical mutations in the LRRK2 gene (Zimprich et al., 2004). Our follow-up study demonstrates the clinical importance of serial contacts and longitudinal evaluation of families in genetic analyses, particularly in age-dependent penetrance disorders.

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Alzheimer disease, association of UBQLN1. *E.R. Martin[1], M.A. Slifer[1], J. Gilbert[1], L. Bertram[3], R.E. Tanzi[3], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 3) Genetics and Aging Research Unit, Massachusetts General Hospital, Charlestown, MA.

Alzheimer Disease (AD) is the most common cause of dementia affecting the elderly. AD is a heterogeneous and complex disease with a strong genetic diathesis. Linkage studies have identified chromosome 9q as a region containing a possible AD candidate gene. Functional protein studies have suggested that the UBQLN1 gene on chromosome 9q may be a likely candidate for a role in late onset Alzheimer disease pathogenesis. A recent family-based study by Bertram et al. NEJM 2005 reported a putative AD risk allele of an intronic single nucleotide polymorphism (SNP) within the UBQLN1 gene. In this study, we sought to assess whether polymorphisms within the UBQLN1 gene are associated with AD in another large family-based data set, as well as an independent case-control data set. We found no significant association of AD with any of the seven SNPs genotyped (including SNP4, previously reported by Bertram et al.) in either the family-based (n= 1161) or case-control (n = 1551) data set. Analyses varying age range and analyses conditional on Apolipoprotein E (ApoE) genotype and sex also revealed no significant associations in either data set. Using age at onset (AAO) as a quantitative trait revealed possible modifying effects associated with two of the SNPs. SNP6 was associated with an earlier AAO in the family based data set (p=0.02), and SNP 4 was associated with a later AAO in the case-control data set (p=0.01). However, the comparator groups in these two analyses were relatively small (n= 24 of 506 affected individuals and n=25 of 546 affected individuals respectively). Our results suggest that UBQLN1 variants do not increase risk for AD in these data. However, there may be a role for an effect modifying age at onset, though the evidence remains limited and inconsistent between the data sets.

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SCN1A may be a susceptibility factor for idiopathic generalised epilepsy. *J. Dean[1], C. Sinclair[2], D. Shaw[2].* 1) Dept Medical Genetics, Argyll House, Aberdeen, United Kingdom; 2) Dept Molecular and Cell Biology, University of Aberdeen, Foresterhill, Aberdeen, United Kingdom.

Idiopathic generalised epilepsy is thought to be a multifactorial disorder, with many genetic aetiologies. Many patients have a distant family history of epilepsy or febrile seizures. GEFS+ (generalised epilepsy with febrile seizures plus, OMIM 604233) is an autosomal dominant form of epilepsy with a variable phenotype in which patients may have febrile seizures in childhood, febrile or non-febrile seizures in later childhood, and partial or generalised seizures as adults. Mutations in SCN1A, SCN1B, SCN2A and GABRG2 have been described in different families. In a study of 156 women with epilepsy, family and personal histories of febrile and non-febrile seizures were noted, suggesting that GEFS+ may be more common that generally thought. To investigate this further, we genotyped these cases and 42 population controls for four SNPs in SCN1A. One non-synonymous coding SNP, T1067A, had a significantly higher frequency in cases (0.51) than in controls (0.33, p=0.001). This suggests that this SNP is a susceptibility factor for epilepsy in this unselected case series from North East Scotland. A previous study in another population suggested that this SNP was not pathogenic (Escayg Am J Hum Genet 2001;68:866-873). Further research is needed to clarify the reasons for these different findings.

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Analysis of opioid receptor variants in the study of pain. *L. Kaplan[1], R.B. Fillingim[2,3], G.N. Verne[3,4], R. Staud[4], M. Agbandje-McKenna[5], C. Haskell-Leuvano[6], M.S. Wood[6], M.C. Moore[6], N.A. Sorensen[6], R. Wu[7], C. Sumners[8], M.R. Wallace[1].* 1) Dept Molec Gen/Microbiol, UF College of Medicine Univ FLorida, Gainesville, FL; 2) Division of Public Health Services, UF College of Dentistry; 3) Malcom Randall V.A. Medical Center in Gainesville, FL; 4) Dept. of Medicine, UF College of Medicine; 5) Dept. of Biochemistry and Molec. Biology, UF College of Medicine; 6) Dept. of Medicinal Chemistry, UF College of Pharmacy; 7) Dept. of Statistics, UF College of Liberal Arts and Sciences; 8) Dept. of Physiology and Functional Genomics, UF College of Medicine.

Baseline pain sensitivity and analgesia response are complex traits, having clear genetic contributions as shown previously with polymorphisms of the OPRM1 and COMT genes (including studies from our group). We have focused our current studies on OPRD1 and OPRK1 (opioid receptors delta and kappa, respectively), which have not yet been extensively studied in this system in humans. Polymorphisms in these two genes are being genotyped in 336 healthy individuals who have undergone experimental pain testing (thermal pain, temporal summation of pain, ischemic pain, and pressure pain). Genotyping of the same variants is also being completed for subjects in two chronic pain populations: fibromyalgia (FMS, n=94) and irritable bowel syndrome (IBS, n=74). Although these syndromes overlap, we have included subjects with just one of these disorders, under strict phenotype criteria. Statistical analysis of allele frequencies in the populations, and associations with phenotypes, is underway. The results will be highly significant for this field regardless of whether the data point to positive or negative associations. We are also analyzing the functional effects of the polymorphisms in OPRD1 and OPRK1, by expressing the variant proteins and testing binding affinity of different selective agonists, and the activation of the receptors. Protein modeling has predicted that at least one of the variants is likely to affect receptor function. We will present our completed data in these novel studies, to shed light on the involvement of the OPRD1 and OPRK1 proteins/pathways in pain.

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The role of hMRE11-hMLH1 interplay in DNA mismatch repair. *F. Zhu[1], N. Zhao[1], F. Yuan[2], G. Li[2], C. Her[1].* 1) SMB, Washington State University, Pullman, WA; 2) University of Kentucky Medical Center, Lexington, KY.

Defective MMR attributes to increased mutation rate and cancer development in humans. Hence, a thorough understanding of human MMR is essential for the development of effective diagnostic and therapeutic strategies. We have recently demonstrated that hMRE11-deficiency led to increased microsatellite instability (MSI) and defective MMR. The involvement of hMRE11 in MMR is presumably mediated by its interaction with hMLH1. In the present study, we investigated the functional implications of the interplay between hMRE11 and hMLH1 in MMR. Briefly, we have created a series of stably transfected 293T cell lines expressing hMLH1 as well as two truncated forms of hMRE11, and nuclear extracts from these cell lines were used to perform an in vitro MMR assay. Our data suggest that overexpression of hMLH1 in MMR-deficient 293T cells could restore a functional MMR, and concomitant expression of the dominant negative hMRE11 aa452-634 but not hMRE11 aa1-634 in these cells caused a significant reduction in both 3 excision and 3 repair activities. Consistent with hMRE11 aa452-634 being the interaction domain for hMLH1, our results suggest that disruption of the interaction between hMRE11 and hMLH1 could have significant effects on MMR. Remarkably, among 33 hMLH1 HNPCC missense mutations that we have analyzed thus far, 31 of them caused significant reduction (> 50%) in their interactions with hMRE11, suggesting disruption of hMRE11-hMLH1 interaction could underlie the functional effects of these mutations in humans. To our surprise, although the hMLH1-complemented 293T cells are MMR proficient, overexpression of hMLH1 in these cells has little, if not none, effect on MSI when compared with that of the parental 293T cells. This result is reminiscent of previous observations that the overexpression of MLH1 could result in a mutator phenotype equivalent to that of MLH1 mutant in yeast. Together, our present study shows that the hMRE11-hMLH1 interaction is essential for functional MMR. In addition, our data suggest that the molecular basis to support functional MMR and microsatellite stability might be mechanistically different in human cells.

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Interaction between GNB3 gene C825T and ACE gene I/D polymorphisms in essential hypertension in Koreans.

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An epistatic interaction effect between G-protein beta-3 subunit (GNB3) C825T and angiotensin I-converting enzyme (ACE) insertion/deletion (I/D) polymorphisms on blood pressure (BP) regulation has been studied. In order to evaluate whether the polymorphisms in the GNB3 gene and ACE gene are associated with essential hypertension (EH) in Koreans, we carried out a case-control study of 691 hypertensive and 763 ethnically- and age-matched control subjects recruited from Cardiovascular Genome Center in Korea. The ACE I/D and GNB3 C825T genotypes were determined by polymerase chain reaction (PCR) and PCR- restriction fragment length polymorphism (RFLP), respectively. The distribution of genotype and allele for the two polymorphisms were not significantly different between the normotensives and the hypertensives. Logistic regression analysis indicated that the GNB3 C825T polymorphism (CC/CT+TT) was strongly associated with EH in the male subjects (OR, 0.656; 95% CI, 0.450-0.955, p = 0.027). In the gene-gene interaction analysis, we found the combined action of GNB3 and ACE genotypes accumulated in the carriers of the ACE D allele and GNB3 CC homozygotes (ID+DD/CC carriers), showing a decrease in risk for hypertension (OR, 0.624; 95% CI, 0.464-0.839, p = 0.0017). In particular, the decrease in the risk for EH was prominent in males (OR, 0.511; 95% CI, 0.315-0.829, p = 0.0061). Stratification analyses of the normotensives and the hypertensives according to the genotypes showed that the levels of triglycerides and fasting glucose were significantly higher in the normotensives carrying the CC genotype in comparison to those carrying T allele (p = 0.036 and p = 0.002, respectively). In our study, we suggest that an interaction between GNB3 C825T and ACE I/D polymorphisms is a positive genetic marker of predisposition for EH in Koreans.

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Characterization of TGFbeta signaling in aortic smooth muscle cells derived from patients heterozygous for R460C TGFBR2 mutation. *S. Duraisamy[1], T. McArthur[1], H. Pannu[1], X.H. Feng[2], D.M. Milewicz[1].* 1) Internal Medicine/Med.Genetics, University of Texas Medical School, Houston, TX; 2) Department of Surgery, Baylor College of Medicine, Houston, TX.

Thoracic aortic aneurysms leading to type A dissections (TAAD) are inherited in an autosomal dominant manner and we have determined that 5% of families with TAAD carry mutations altering arginine 460 in the intracellular kinase domain in transforming growth factor- receptor type II (TGFBR2). We transfected cDNA for the mutant TGFBR2 into cells (DR-26) lacking the TGFBR2 to determine if the mutation disrupted the kinase activity of the receptor, which indicated that the mutation prevented downstream signaling of the receptor. Aortic smooth muscle cells (SMCs) explanted from 2 unrelated patients heterozygous for R460C TGFBR2 mutations were studied; along with two control aortic SMCs. Analysis of patients cells Smad 2/3 phosphorylation in response to TGFbeta indicated that in the absence of TGFbeta the control cells showed no p-Smad, whereas the patients cells surprisingly showed p-Smad in the absence of TGFbeta, suggesting the TGFbeta pathway was stimulated in the absence of TGFbeta signaling. Both control and patients cells phosphorylated Smad 2/3 in the presence of TGFbeta. These results were supported by immunofluorescence analysis showing increased p-Smad localization in the nucleus of SMCs derived from the patient heterozygous for a TGFBR2 mutation when compared to control in the absence of TGFbeta. Furthermore, RNA was isolated from control and TGFbeta mutant SMCs after serum starvation and the expression of TGFbeta targeted genes was examined by quantitative RT-PCR. The expression of plasminogen activator inhibitor-I ($p<0.009$), connective tissue growth factor ($p<0.02$) was increased and matrix metalloproteinase 2 ($p<0.0009$) was decreased in TGFBR2 mutant cells when compared to control cells, supporting basal stimulation of the TGFbeta pathway. In summary, SMCs heterozygous for R460C TGFBR2 mutation demonstrate paradoxical activation of TGFbeta signaling in the absence of ligand, data that potentially provides insight into a pathway leading to aortic aneurysms and dissection.

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C-reactive protein (CRP) gene variation is associated with serum CRP in dialysis population. *L. Zhang[1], W. Kao[1], Y. Berthier-Schaad[1], N. Fink[1], N. Powe[1], M. Klag[1], M. Smith[2], J. Coresh[1].* 1) Epidemiology, Johns Hopkins University, Baltimore, MD; 2) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.

Elevated serum CRP level is an important risk factor for cardiovascular disease in both the general population and in high-risk groups, such as dialysis patients. Although previous studies have demonstrated association between CRP genetic variation and serum CRP levels in the general population, it is not known whether the same associations would be detectable in the dialysis population, where CRP levels are much higher than those in the general population due to dialysis-related factors. We examined this association in 417 white and 196 African-American incident dialysis patients with mean age 58 yrs. Bins of SNPs in strong linkage disequilibrium (r2>0.60) were identified by SeattleSNPs program and seven tagging SNPs (minor allele frequency>5%) were selected to cover all bins of the gene. Genotyping was conducted using the Illumina platform. High sensitivity CRP and IL-6 were measured at the beginning of the dialysis session. Haplo.stats was used to examine the association of haplotype with log-transformed CRP. Haplotypes with <5% frequency were combined. An a priori specified additive genetic model was assumed. Median CRP level was 3.96 mg/l and 3.30 mg/l in whites and African Americans, respectively. Four major haplotypes were identified in whites, and 7 in African Americans. In both whites and African Americans, significant association between haplotypes and serum CRP levels were observed, even after adjusting for age, gender, body-mass index, systolic blood pressure, serum albumin and total cholesterol levels, and co-morbidities. A permutation global test of all haplotypes with CRP resulted in a p-value of 0.08 in whites and 0.0003 in African Americans. Compared to the common haplotype, haplotype 3 was associated with higher levels of CRP in whites while haplotypes 4 and 6 were associated with lower levels of CRP in African Americans. The association was independent of baseline IL-6 level. Variation in the CRP gene is associated with serum CRP levels in the dialysis population.

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Genetic Complexities of Molecular Diagnosis for Ventricular Arrhythmias. *N.H. Bishopric, T. Miller, S. Cocilova, L. You, H. Travers, S. Elliott, R. Myerburg, L. Baumbach.* Miller School of Medicine, University of Miami, Miami , FL.

Molecular diagnosis of Long QT syndrome (LQTS) and related arrhythmogenic disorders remains highly complex, due to numerous private mutations, variants of unknown significance, and low penetrance alleles. We report our experience performing mutation screens of five LQTS-associated disease genes (KCNQ1, KCNH2, KCNE1, KCNE2, SCN5A) in probands referred from a tertiary care hospital for clinically suspected LQTS or Jervell-Lange-Nielsen syndrome (n=78)or pharmacologically-induced (drug induced; DI-LQT) and torsade (n=4). Mutation screening for all genes was conducted using exon-specific PCR, SSCP, and DNA sequencing of all SSCP variants. DNA variants were reviewed for relative frequency and possible disease association using mutation databases and published literature. Presumed disease-causing mutations were detected in twenty-two (28%) suspected LQTS patients. Twelve of these were in KCNQ1; five were in KCNH2; the remaining five mutations were distributed across the three remaining loci. Of the 17 KCNQ1 and KCNH2 mutations, six were novel. Coinheritance of mutations with disease phenotype was proven in 6/7 families analyzed. It is of interest that four of the twelve (33%) cases with KCNQ1 mutations had a second mutation (D85N) in KCNE1, the beta-subunit of KCNQ1. In addition to these deleterious mutations, two synonymous and fourteen non-synonymous SNPs were also detected. Each LQTS proband displayed numerous SNPs in all five genes, including at least one SCN5A variant. Of the four DI-LQT patients, only one had a known pathogenic mutation (KCNQ1, G189R), while two had rare and/or novel SNPs (KCNH2 1809 C>T; KCNQ1 I93I + SCN5A 3183G>A). These combined results suggest that with increased availability of genetic testing for LQTS, bona fide disease-causing mutations are likely to be identified in fewer than 30% of all patients referred with suspected LQTS, and that the majority of patients will harbor novel mutations or gene variants of unknown significance. Our data suggests that additional functional and epidemiological studies are warranted to establish better predictive genotype-phenotype correlations.

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Estrogen Metabolism Gene Polymorphisms and Hypertension in Women. *S. Colilla[1], S. Hollenberg[2], A. DeMichele[1], G. Bunin[1], R. Schinnar[1], B. Strom[1], T. Rebbeck[1].* 1) Dept Biostatistics & Epidemiol, Univ Pennsylvania, Philadelphia, PA; 2) UMDNJ-RWJMS, Camden, NJ.

Recent studies suggest that the protective effects of estrogen on cardiovascular disease may be due in part to intracellular estradiol metabolites. We hypothesized that polymorphisms in estrogen metabolism genes would affect the prevalence of hypertension in women. Using a randomly selected group of women from the Delaware Valley who were ascertained as controls for a case-control study for breast and endometrial cancer, polymorphisms *CYP1A2**1F, *COMT* Val158Met, *CYP1B1**3, *CYP1B1**4, *CYP3A4**1B, and *SULT1E1* 5UTR were genotyped from DNA extracted from buccal swab samples provided by 1764 women. Multivariate logistic regression was used to estimate the association between each genetic polymorphism and the prevalence of hypertension. None of the polymorphisms showed a significant association with hypertension in women of European ancestry, after adjustment for age, body mass index, and diabetes. Adjustment for menopausal status, hormone replacement therapy use, income and education did not measurably change the results. The Met (M) allele, which has been shown to reduce COMT activity three-fold, was significantly associated with a decreased prevalence of hypertension ($p = 0.04$) in women of African ancestry. African-American women who were heterozygotes (V/M) were 28% less likely to be hypertensive than African-American women who were homozygotes of ancestral allele (V/V) [OR = 0.77, 95% C.I. (0.50, 1.19), $p = 0.24$]. African-American women who were homozygous M/M were half as likely to be hypertensive as women with the V/V genotype [OR = 0.50, 95% C.I. (0.26, 0.96), $p = 0.04$]. The Met polymorphism which reduces COMT activity may cause higher intracellular levels of estradiol and explain the reduction of hypertension in African-American women with M/M genotype. Further analysis may clarify whether differences found between the two racial groups are due to residual confounding or effect modification between the *COMT* polymorphism and another factor. Nonetheless, we provide evidence that the *COMT* genotype is associated with hypertension in African-American women.

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Genome-wide scan for hypertension: The National Millennium Project in Japan. *T. Miki[1], Y. Tabara[2], J. Nakura[1], K. Kohara[1].* 1) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Environment Health & Social Medicine, Ehime Univ Sch Medicine, Ehime, Japan.

In 2000, national cooperative projects, under the banner of "Millennium Projects", were started in Japan. The identification of genetic variations linked to the development of hypertension is one of the leading missions of the Millennium Project. The whole-genome case-control analyses, using 100,000 SNP markers and 16,000 microsatellites, have been performed as a 1st screening. The SNPs were selected from the JSNP database, a database of SNPs in Japanese population (http://snp.ims.u-tokyo.ac.jp/). The microsatellites were assigned less than 600 kb apart. The case subjects (n=188) fulfilling the following criteria were recruited from Japan nationwide: male, BMI <= 25 kg/m2, SBP => 160 mmHg and/or DBP => 100 mmHg or under anti-hypertensive treatment, age of onset was between 30 and 59 (y.o.), and having family history of hypertension within parents and siblings. The contrastive patients with other four diseases were served as the control subjects (n=752). In the first-screening, 21,602 SNPs showed probability values less than 0.05 in the 2X2 and/or 2X3 chi-squared analyses. In the 2nd screening, 1,800 SNPs were analyzed in other 752 hypertensive cases and 752 normotensive controls, and 212 SNPs remained statistically significant. To further clarify the susceptible SNPs, 3rd screening was carried out using other case-control (619/1,406) subjects. Of 212 SNPs, 4 SNPs showed probability values less than 0.05 (0.003 to 0.035) in the comparison of allele frequency. The odds ratios of these SNPs were between 1.18 and 1.23 in the allelic model. In the micorosatellite markers analysis, 957 loci showed significant associations in the 1st screening. Of these markers, 257 loci remained statistically significant in the 2nd screening and the most powerful association was observed in chromosome 1 (p=1.2x10-23). To further narrow the susceptible loci and to identify the tag-SNPs, the haplotype analysis is now carrying on. The validity of these tag-SNPs will be verified using the 12,000 population samples.

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A SNP association study suggests the LRP8/ApoER2 gene on chromosome 1p34-36 to be a susceptibility gene for coronary artery disease (CAD) and myocardial infarction (MI). *S.Q. Rao[1,2,3], G.Q. Shen[1,2,3], L. Li[1,2,3], E.J. Topol[1,2,3], Q.K. Wang[1,2,3].* 1) Department of Molecular Cardiology; 2) Department of Cardiovascular Medicine; 3) Center for Cardiovascular Genetics; Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

Our genome-wide linkage scan of 428 nuclear families in the U.S. identified a significant genetic locus for susceptibility to MI on chromosome 1p34-36. Here we describe a population-based case-control association study for analyzing candidate genes at the locus. The 382 cases consist of the well-characterized CAD/MI probands from each family with DNA available, and the 315 controls are individuals without detectable stenosis by coronary angiography. Sixteen candidate genes were characterized, including the connexin37 gene (GJA4), the endothelin-converting enzyme-1 (ECE1), and the low-density lipoprotein receptor-related protein 8 (LRP8, or ApoE receptor 2). Genotyping of SNPs (single nucleotide polymorphisms) was carried out using the 5' nuclease allelic discrimination assay with an ABI Prism 7900HT Sequence Detection System and the genotyping data yielding positive associations were verified by direct DNA sequence analysis. The allelic associations of SNPs with CAD/MI were evaluated using Pearsons Chi-square test. Only one of the 16 candidate genes, LRP8, showed significant association with CAD and MI. Three SNPs in LRP8 are associated with MI (P-value, from 0.03 to 0.001), and two SNPs showed association with CAD (P-value from 0.02 to 0.003). These results suggest that LRP8 is a susceptibility gene for CAD and MI.

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A systemic SNP survey of 123 candidate genes from important pathways for genetic susceptibility of coronary atherosclerosis in Chinese populations. *Y. Wang[1], Y. Wang[2], Y. He[2], H. Wang[1,3], L. Xu[1], Y. Liu[1,3], Y. Wang[1], W. Sun[1], Y. Wang[1], X. Xiong[1], A. Sun[4], J. Ge[4], M. Xiong[1,2], L. Jin[1,2], W. Huang[1].* 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China; 3) Shanghai South Gene Technology Co., Ltd., Shanghai, China; 4) Zhongshan Hospital, Fudan University, Shanghai, Chin.

It has been widely accepted that the mechanism of atherosclerosis is mainly due to response to injury. This complex and progressive procedure involved many biological pathways, such as oxidant-antioxidant, inflammation, lipid metabolism, and coagulation. Despite observations on correlations of individual genes with atherosclerosis in vitro and in vivo, systemic association studies involving all genes from each pathways and many genes from multiple pathways have yet been reported. Here we offer a systemic SNP survey of 123 candidate genes involved in multiple pathways which have been implicated in the pathogenesis of coronary atherosclerosis in Chinese populations. In this study, we typed overall 1,518 haplotype tagging SNPs from 123 candidate genes in 916 samples (492 atherosclerotic cases and 424 controls). The result demonstrated that totally 116 SNPs and 41 haplotypes representing 38 susceptive genes were observed to be highly associated with coronary atherosclerosis. The association of each individual pathway with coronary atherosclerosis was also evaluated. Furthermore, we showed that there exist a few significant interactions between the genes within and between pathways.

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The Promoter Polymorphisms of HMOX1 and Cardiovascular Disease. *J. Pohorence Ferguson[1], G.E. Cooke[2], P.F. Binkley[2].* 1) Dept Pathology, Ohio State University, Davis Heart and Lung Research Institute, Columbus, OH; 2) Dept Cardiovascular Medicine, Ohio State University, Davis Heart and Lung Institute, Columbus, OH.

A loss of heme oxygenase (HO-1) activity increases susceptibility to oxidative damage and has been associated with cardiovascular disease. Transcriptional activity of HMOX1, the gene encoding HO-1, is reduced by a promoter microsatellite polymorphism, while a promoter T(-413)A polymorphism has been found to increase transcriptional activity. Sequencing analysis was performed on a total of 676 subjects, 531 Caucasians and 145 African Americans, to genotype for the HMOX1 promoter polymorphisms. The African American race was significantly associated with the L allele and LL genotype of the microsatellite polymorphism ($p < 0.001$), and the SNP-413 T allele and TT genotype ($p < 0.001$). Further work was done to explore the relationship between the HMOX1 promoter polymorphisms and cardiovascular disease, especially in the African American population. In 138 patients who underwent percutaneous transluminal coronary angioplasty (PTCA), the LL genotype was significantly associated with a history of coronary artery disease ($p = 0.04$), and the T allele was significantly associated with a history of hypertension ($p = 0.03$) regardless of race. The 55 African American PTCA patients had a 3.75-fold increased odds of having the LL genotype ($p = 0.009$). In 281 patients having congestive heart failure, 49 African American patients had a 2.26-fold increased odds of having the LL genotype ($p = 0.01$). A subset of 71 patients who received heart transplants had a significant association between the LL microsatellite allele genotype and the AA SNP-413 genotype ($p < 0.001$). Quantitative PCR analysis for HMOX1 expression was performed on tissue samples from the explanted hearts of 38 transplant recipients. Bar graphs of the mean HMOX1 expression show the SS,TT genotype combination had the highest expression, although this was not statistically significant. The results of this study reveal the African American population may be susceptible to cardiovascular diseases involving oxidative stress due to a decrease in HO-1 activity resulting from the presence of the promoter polymorphisms.

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Identification of Down Syndrome Heart Defect Candidate Gene. *L.J.H. Bean[1], S.F. Freeman[1], K.J. Dooley[2], T.C. Rosser[1], C. Oxford-Wright[1], C. Koller[3], G. Capone[3], S.L. Sherman[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Sibley Heart Center, Department of Pediatrics, Emory University, Atlanta, GA; 3) Division of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD.

Individuals trisomic for chromosome 21 exhibit a wide range of phenotypes known as Down syndrome (DS). DS is mainly characterized by mental retardation and clinical features that include hypotonia, abnormalities of the face, hands, and feet. Other variable phenotypes associated with DS may include congenital heart disease (CHD), digestive tract abnormalities, congenital cataracts, or leukemia. The prevalence of DS, approximately 1 in 600 to 1 in 1000 live births, makes this syndrome the most commonly identified form of mental retardation. Compared to the general population, individuals with DS are at a 2000-fold increased risk for complete atrioventricular septal defects (AVSDs), a severe form of CHD. The purpose of this study is to identify genetic variants on chromosome 21 that contribute to CHD susceptibility using a candidate gene approach in a DS population with complete AVSDs. We have asceratined the largest reported collection of DNA on complete AVSD DS cases and their parents. This study is currently being expanded from 50 to 400 individuals with DS and AVSD to facilitate molecular studies and gene identification, in part by patient identification and recruitment from the Sibley Heart Center at Childrens Healthcare of Atlanta. Studies done in the mouse indicate that several genes within the candidate region are differentially expressed during heart development. We hypothesize that genes expressed in fetal, but not adult, heart may play a role in the etiology of congenital heart defects. The human heart expression patterns of several interesting candidate genes in a 10Mb DS CHD critical region on chromosome 21 have been determined. Taking advantage of the vast array of genomic resources available for chromosome 21, we will use molecular approaches to identify candidate genes for DS CHDs within a 10 Mb heart defect critical region and elsewhere in the genome.

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Epicardial coronary artery spasm and microvascular angina are differentially influenced by *PON1* A632G polymorphism in the Japanese. *G. Koike, J. Mashiba, H. Kamiunten, K. Sunagawa.* Dept of Cardiovascular Med, Kyushu Univ Grad Sch Med Sci, Fukuoka, Japan.

Background: Ethnicity and smoking are well-known risk factors for the pathogenesis of coronary vasospasm. Oxidative stress induced by smoking plays a crucial role in increasing blood vessel tonus resulting in coronary vasospasm. However, smoking is not enough to account for the pathogenesis of coronary vasospasm, indicating that genetic factors are strongly involved. Therefore, the aim of this study is to elucidate oxidative stress related genetic factors responsible for the pathogenesis of coronary vasospasm. **Methods:** One hundred sixty two epicardial coronary artery spasm patients (LASs), 61 microvascular angina patients (MVAs), and 61 non-responders (NRs) diagnosed by acetylcholine provocation test were ascertained. After collecting genomic DNA from these patients, 4 polymorphisms of oxidative stress related genes, *CYBA* (cytochrome b-245, alpha polypeptide gene) C242T and A640G, *PON1* (paraoxonase 1 gene) A632G, *PLA2G7* (phospholipase A2 group VII gene) G994T were determined. Using clinical and genotyping information, statistical analysis was carried out. **Results:** Allele frequency of *PON1* 632-G was significantly higher in both the LAS with dominant fashion (OR 2.24, 95% CI 1.04-4.82, p=0.035) and the MVA with recessive fashion (OR 2.22, 95% CI 1.08-4.58, p=0.030) compared to the NR. This association was strongly influenced by gender only in the MVA. There were no significant associations between other polymorphisms and coronary vasospasms. In addition, allele frequency of *PON1* 632-G in the Japanese was higher than that in the Caucasian. **Conclusion:** There was a significant association between *PON1* A632G polymorphism and MVA as well as LAS. But the way of this impact on LAS and MVA was different in the Japanese.

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Impact of prenatal and/or early postnatal environments on the cardiovascular stress response is modified by genes on rat chromosome 20. *J. Berube[1], L. Sedova[2], M. Pravenec[3], V. Kren[2], P. Hamet[1], Z. Pausova[1,4].* 1) Centre hospitalier de l'Université de Montréal, Canada; 2) Charles University and; 3) Academy of Sciences, Czech Republic; 4) Brain & Body Centre, University of Nottingham, UK.

Prenatal and/or early postnatal environments may play an important role in programming cardiovascular health in later life. This effect can be modified by the individuals genetic makeup. Augmented reactivity and delayed cardiovascular recovery are predictors of cardiovascular morbidity and mortality. The aim of the present study was to investigate in the rat whether litter size and parity (as mother-derived early-life environments) determine the offsprings cardiovascular response to stress, and if the effect is modified by genes within a specific segment of rat chromosome 20 (RNO20). At 16 weeks of age, systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) were measured by telemetry during and after 30-min immobilization stress. Spontaneously hypertensive rats (SHR) and its congenic strain, SHR.1N, were studied. SHR.1N differs from SHR by only a segment of RNO20, which has been transferred onto the SHR background from the normotensive Brown Norway rat. Two-way ANOVA for repeated measures with parity (primipara vs. multipara), litter size (small 9 pups vs. large 9 pups) and strain (SHR vs. SHR.1N) as main factors was used. During immobilization, SBP, DBP, and HR increased in both strains, but significantly more in SHR.1N. This effect of the RNO20 segment was modified by parity but not by litter size. Thus, when compared to rats born to multiparas, SHR.1N from primiparas showed higher SBP, DBP, and HR elevations, whereas SHR born to primiparas demonstrated lower elevations. During the 30-min post-stress period, SBP, DBP and HR decreased similarly in all animals, except for SHR.1N born to primiparas or originating from large litters that showed significantly delayed post-stress recovery of SBP, DBP, and HR. Our results indicate that mother-derived early-life environments, such as parity and/or litter size, significantly influence both reactivity and recovery of the cardiovascular system from stress in adulthood. This influence is modified by a gene(s) within the RNO20 segment.

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In vivo and in vitro studies support that a new splicing isoform of OLR1 gene is protective against acute myocardial infarction. *R. Mango[1], S. Biocca[1], F. del Vecchio[1], F. Clementi[1], F. Sangiuolo[1], F. Amati[1], A. Filareto[1], S. Grelli[1], P. Spitalieri[1], I. Filesi[1], C. Favalli[1], R. Lauro[1], J.L. Mehta[2], F. Romeo[1,2], G. Novelli[1,2].* 1) Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, University of Tor Vergata,Rome, Italy; 2) Division of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1), encoded by the OLR1 gene, is a scavenger receptor that plays a fundamental role in the pathogenesis of atherosclerosis. LOX-1 activation is associated with apoptosis of endothelial cells, smooth muscle cells (SMCs) and macrophages. This process is an important underlying mechanism that contributes to plaque instability and subsequent development of acute coronary syndromes. Independent association genetic studies have implicated OLR1 gene variants in myocardial infarction (MI) susceptibility. Since single nucleotide polymorphisms (SNPs) linked to MI are located in intronic sequences of the gene, it remains unclear as to how they determine their biological effects. Using quanitative real-time PCR and minigene approach, we show that intronic SNPs, linked to MI, regulate the expression of a new functional splicing isoform of the OLR1 gene, LOXIN, which lacks exon 5. Macrophages from subjects carrying the non-risk disease haplotype at OLR1 gene have an increased expression of LOXIN at mRNA and protein level, which results in a significant reduction of apoptotis in response to oxLDL. Expression of LOXIN in different cell types results in loss of surface staining, indicating that truncation of the C-terminal portion of the protein has a profound effect on its cellular trafficking. Furthermore, the pro-apoptotic effect of LOX-1 receptor in cell culture is specifically rescued by the co-expression of LOXIN in a dose-dependent manner. The demonstration that increasing levels of LOXIN protect cells from LOX-1 induced apoptosis set a groundwork for developing therapeutical approaches for prevention of plaque instability.

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The Bardet-Biedl protein BBS2 has a role in mitotic spindle dynamics and chromosome alignment. *J. Wei[1,2], D.Y. Nishimura[2], Q. Qian[2], C. Searby[1,2], M. Andrews[1,2], K. Bugge[2], A. Ferguson[2], A. Fedler[2], A. Loux[2], S.R. Patil[2], V.C. Sheffield[1,2].* 1) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Bbs2$^{-/-}$ mice exhibit a typical *Bbs*$^{-/-}$ phenotype: retinal degeneration through apoptosis, failure of spermatozoa flagella formation, obesity associated with increased food intake, and development of renal cysts. Previously, we reported that rhodopsin in some *Bbs2*$^{-/-}$ photoreceptor cells remained in the cell bodies rather than being transported to the outer segments, which normally occurs via microtubule-dependent transport. This mislocalization of rhodopsin in the cell body suggests that BBS2 plays a role in microtubule-directed transport. Therefore, we examined the localization of BBS2 to explore whether BBS2 is a microtubule-associated protein. By immunofluorescent imaging of both wt and *Bbs2*$^{-/-}$ renal cells stained with antibodies directed against tubulin and BBS2, we have shown that BBS2 is a microtubule-associated protein. Based on this observation, we examined the overall structure of the mitotic spindle. We observed that ~10% of *Bbs2*$^{-/-}$ renal cells at metaphase showed defects in chromosomal alignment on the metaphase plate. One or few chromosomes were found to attach to either one pole or two poles of the mitotic spindle in some *Bbs2*$^{-/-}$ renal cells. Interestingly, once the chromosome moved away from the mitotic spindle, the cells proceeded with the division. Further studies revealed that more than 20% of *Bbs2*$^{-/-}$ fibroblasts exhibited aneuploidy with both losses (38 to 39 chromosomes) and gains (41 chromosomes) compared to mouse euploidy (40 chromosomes). These data indicate that BBS2 contributes to mitotic spindle dynamics and mutations in BBS2 result in increased levels of chromosomal inbalances in somatic cells.

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Haplotypes of DAZL Are Associated with Susceptibility to Spermatogenic Failure in the Taiwanese Population.

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Background: Genetic factors have been associated with impaired sperm production in humans. DAZL is a germ-cell specific autosomal gene and is a strong candidate gene for human spermatogenic failure. Methods: DAZL was genotyped in 231 men with spermatogenic failure and 191 men with proven fertility. We carried out case-control analysis to identify the DAZL haplotypes which are associated with spermatogenic failure. Results: Five SNPs were identified: 260A>G (Thr12¡÷Ala) in exon 2, 386A>G (Thr54¡÷Ala) in exon 3, 520+34c>a in intron 4, 584+28c>t in intron 5, and 796+36g>a in intron 7. Of the five SNPs, three were novel: 520+34c>a, 584+28c>t, and 796+36g>a. The linkage disequilibrium (LD) map patterns were different for infertile men and control subjects. Four major haplotypes constituted 88.0% of the patient population, while three major haplotypes constituted 87.0% of the control population. Of all haplotypes studied, three haplotypes were over-transmitted in patients with severe spermatogenic failure, while two were under-transmitted in these patients. Conclusions: Different LD maps for fertile and infertile men indicate distinct genetic backgrounds for these two groups of subjects. The haplotyes which were over-transmitted in the infertile men conferred susceptibility to spermatogenic failure, while under-transmitted haplotypes were protective against spermatogenic failure. Our study suggests autosomal DAZL gene is a strong candidate gene for human spermatogenic failure.

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POLYMORPHISMS IN THE TUMOR NECROSIS FACTOR-ALPHA GENE IN TURKISH WOMEN WITH PRE-ECLAMPSIA AND ECLAMPSIA. *A. Pazarbasi[1], M. Kasap[1], A.I. Guzel[1], H. Kasap[1], M. Onbasioglu[2], B. Ozbakır[2], F.T. Ozgunen[2].* 1) Medical Biology and Genetics, University of Cukurova, Adana, Turkey; 2) Obstetrics and Gynecology, University of Cukurova, Adana, Turkey.

The genetic background predisposing pregnant women to pre-eclampsia/eclampsia (PE/E) is still unknown. Three recent reports indicated that the tumor necrosis factor-alpha gene (TNF-alpha) could be involved in susceptibility to PE/E. TNF-alpha is a potent modulator of immune and inflamatory responses, and has been implicated in a variety of diseases, including PE/E. 308G/A and 850 C/T polymorphisms within the TNF-alpha gene promoter, has been associated with negative outcome in some diseases, including PE. The association of the TT allele with Eclampsia is unknown. The aim of this current study was to investigate whether there is an association between the TNF-alpha-308 and 850 polymorphisms and PE or eclampsia. In this study, 12 cases of eclampsia, 103 cases of PE and 46 normotensive control cases were genotyped for the TNF-alpha-308 and 850 polymorphisms. The uncommon allele (TNF2) of 308G/A polymorphism in the promoter region of the TNF-alpha gene has been reported to be increased in a variety of diseases, including eclampsia. We found a significant difference between the TNF2 allele frequencies of eclamptic, pre-eclamptic and normotensive controls. TNF2 polymorphism frequency was significantly higher among those eclamptic and pre-eclamptic (control: 6.5%, PE: 13.6%, E: 33.3%). A significantly different genotype distribution of 850C/T polymorphism observed between the PE/E and control groups, with the frequency of the variant T allele being significantly reduced in the preeclamptic (17.5%) and eclamptic group (16.6%) when compared with the control group (24%). We conclude from this study that the TNF2 allele contributes to the occurence of eclampsia and pre-eclampsia and the T allele of the TNF-alpha gene may modify individual PE/E risk, being protective against the development of the complication.

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Brain derived neurotrophic factor (BDNF) haplotypes are associated with opioid addiction. *R. de Cid[1,2], F. Fonseca[3], M. Bayés[1,2], M. Gratacòs[1,2], R. Martín-Santos[1,4], X. Estivill[1,2,5], M. Torrens[3].* 1) Genes & Disease Program, Center for Genomic Regulation, Barcelona, Catalonia, Spain; 2) CeGen, Spanish Genotyping Centre, Barcelona, Catalonia, Spain; 3) Institut dAssistencia Psiquiàtrica, Salut Mental i Toxicomanies (IAPS), Hospital del Mar, Barcelona, Catalonia, Spain; 4) Unitat de Recerca en Farmacologia, Institut Municipal dInvestigació Mèdica (IMIM), Barcelona, Catalonia, Spain; 5) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Brain derived neurotrophic factor (BDNF) signaling pathway have been shown essential for opiate-induced molecular adaptation of the noradrenergic system in murine models. Drug induced adaptations in reward circuits have been described as a form of synaptic plasticity. BDNF studies in drug abuse have revealed modest associations between substance abusers and controls, but until now none have assessed effect of BDNF in opiate addiction. Genetic variability in BDNF could be related to poor response to Methadone Maintenance Treatment (MMT) in opiate addicted patients. We carried out a case-control study of opioid dependent subjects consecutively admitted into a MMT, matched by gender and age with naïve controls. After 7-month follow-up patients were divided into responders and nonresponders to MMT, based on illegal opiate consumption and retention in treatment. Substance and non-substance use disorders diagnoses were obtained mean PRISM-IV. Genetic variability in BDNF was addressed by SNPlex with 44 SNPs along BDNF gene. Nineteen of 44 assessed SNPs were informative in our sample. Five out nineteen in the BDNF gene showed allelic differences with statistical nominal differences among opiate-addicted-patients and healthy-controls ^{2} test uncorrected $p<0.001$). None of the observed allelic differences itself resisted the permutation test for significance. Simple regression analysis for genotype distribution shows that one of these variants was associated with opiate addiction under a recessive model. Genetic variability at BNDF shows association with opiate addiction, but no with MMT response. Supported by Marato-TV3 (10810) and RGPG-FIS (G03/184).

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Neurocognitive trait components of schizophrenia - an association study in twins. *O.P.H. Pietilainen[1], T. Paunio[1], A. Loukola[1], A. Tuulio-Henriksson[2], W. Hennah[1], J. Turunen[1], J.O. Peltonen[1], K. Silander[1], J. Lonnqvist[2], J. Kaprio[3], T.D. Cannon[4], L. Peltonen[1,5].* 1) National Public Health Institute, Dept of Molecular medicine, Biomedicum, P.O.Box 104, FIN-00251 Helsinki, Finland; 2) National Public Health Institute, Dept of Mental Health and Alcohol Research, Helsinki, Finland; 3) University of Helsinki, Dept of Public Health, Helsinki, Finland; 4) UCLA, Depts of Psychology and Psychiatry and Biobehavioral Sciences, Los Angeles, USA; 5) University of Helsinki, Dept of Medical Genetics, Helsinki, Finland.

Susceptibility to schizophrenia (SZ) is influenced by numerous genes affecting a variety of brain functions. Some of these may be inherited in a predisposing configuration without resulting in a clinical phenotype, however affecting the function of the relevant brain systems. Twins represent ideal study subjects to address the genetic background of neurobehavioral traits disturbed in SZ as they share the same environment during the fetal period and early years of their lives. We tested several variables measuring neurocognitive functions in a Finnish twin study sample consisting of 58 SZ twin pairs (8 concordant, 50 discordant), and 62 control twin pairs matched for age, sex and demographics. We analyzed 27 SNPs in three candidate genes reported to be associated with SZ: NRG1, DTNBP1, and AKT1. We used linear regression with age, gender, presence of psychosis, zygosity, and the co-twin SZ status as covariates. Among the tested neuropsychological variables, one related to attention and visual motor tracking showed association with a variant of NRG1 ($p=0.0008$). Suggestive association was observed for visual working memory and visual attention with a variant in DTNBP1 ($p=0.003$ and $p=0.008$). This study highlights the importance of quantitative neurocognitive features as endophenotypes for SZ in the characterization of the genetic background of this disorder. The study further supports the role of NRG1 in the genetic etiopathogenesis of SZ and, furthermore, elucidates the putative brain systems that seem to be primarily affected. We are currently confirming these results in a large nationwide SZ study sample.

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Craniofacial features of BBS patients and Bbs4 null mice. *P.L. Beales[1], E.R. Eichers[2], P. Hammond[3], M. Garcia[2], M.J. Justice[2], J.R. Lupski[2,4,5].* 1) Molecular Medicine Unit, Inst of Child Health, University College London, London, UK; 2) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Biomedical Informatics Unit, Eastman Dental Institute for Oral Health Care Sciences, University College London, London, UK; 4) Dept of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Texas Children's Hospital, Houston, TX.

The oligogenic disorder Bardet-Biedl syndrome (BBS) is characterized by clinical heterogeneity and complex inheritance. Features of BBS include mental retardation, polydactyly, obesity, hypogenitalism, renal abnormalities, retinal dystrophy, and several secondary features, although the phenotype is variable between patients. To analyze the contribution of one gene, *Bbs4*, to the BBS phenotype in mouse we created a knockout mouse line lacking expression of *Bbs4*.

We have demonstrated previously that BBS patients may have a characteristic face. Three-dimensional surface modeling of the faces of patients has suggested that BBS features include a wider, rounder face with a shorter, flattened nose, and retrognathia.

To further explore the characteristic face of patients, we examined a mouse model of BBS for a craniofacial phenotype. By direct observation it could be discerned that null mice appeared to have a distinct face as compared to their littermates. Thus, we subjected our mice to three-dimensional soft-tissue craniofacial scanning. Analysis of data points from key landmarks on the collected images indicate that the overall distance between several of the landmarks was significantly different in the null mice when compared to their littermates. For example the mean distance from the nasion to the subnasale (nsn) was significantly shorter (i.e a shorter snout) in null animals. Analyses of comparable nsn distances in 37 human BBS patients revealed females had a significant shortening but in males this distance was longer. However, when viewed as ratios (ngn:nsn) the overall human facial length was similar to that (vgn:nsn) for null mice. These results suggest that BBS genes play a significant role in midface development in mammals.

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Behavioral analyses of *Bbs4* mice. *E.R. Eichers[1], N. Katsanis[5,6], P.L. Beales[7], R. Paylor[1,3], J.R. Lupski[1,2,4].* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Division of Neuroscience, Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston, TX; 5) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 6) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 7) Molecular Medicine Unit, Institute of Child Health, University College, London, UK.

Bardet-Biedl syndrome (BBS) is a rare oligogenic disorder exhibiting clinical heterogeneity and complex inheritance. Although the BBS phenotype is variable both between and within families, the syndrome is characterized by mental retardation, polydactyly, obesity, hypogenitalism, renal abnormalities, retinal dystrophy, and several secondary features. Eight genes mutated in BBS patients have been identified. To investigate the etiopathogenesis of BBS, we created a mouse null for one of the Bbs genes, *Bbs4*, to analyze the contribution of one gene to the pleiotropic BBS phenotype in mouse.

One phenotype more recently explored clinically in BBS patients is that of behavior anomalies. Although the behavioral characteristics of BBS patients are variable in both penetrance and expressivity, patients have been noted to have difficulty with thought and attention span, social problems, and to be withdrawn, anxious and depressed. In order to evaluate our mouse model for any behavioral phenotypes, we performed a battery of tests on null mice and their wild-type littermates. Our test results indicate that, in a gender-independent manner, the *Bbs4* null mice have significantly higher levels of anxiety than their wild-type littermates, as evidenced by both open-field locomotor and light-dark exploration experiments. Additionally, our knockout mice are significantly less socially dominant than their wild-type littermates. These data suggest that alterations in BBS4 may contribute to behavioral anomalies observed in some BBS patients.

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Detecting chromosomal abnormalities in individuals with autism using karyotyping, FISH, and high-density cDNA microarrays. *T.H. Wassink[1], V.C. Sheffield[2], E.R. Westin[3], E. Ashley[4], J. PIven[4], S.R. Patil[5].* 1) Department of Psychiatry, UIA Carver College of Medicine, Iowa City, IA; 2) Department of Pediatrics and the Howard Hughes Medical Institute, UIA Carver College of Medicine, Iowa City, IA; 3) Inter-Departmental Genetics Program, UIA, Iowa City, IA; 4) Neurodevelopmental Disorders Research Center and Department of Psychiatry, University of North Carolina, Chapel Hill, NC; 5) Department of Pediatrics, UIA Carver College of Medicine, Iowa City, IA.

As part of an ongoing study to evaluate the genetics of autism, we have studied 127 individuals cytogenetically with karyotyping and focused FISH analyses (subtelomeres: n=119; 15q11.2q13: n=49; 22q11.2: n=20). Thus far, 7/127 samples (5.5%) have had a chromosomal abnormality. Four of the abnormalities were autosomal [del(2)(q37.1q37.3); inv(8)(p23.2q13.1); inv dup (15q) supernumary marker; dup(15q11.2q13)] and three were sex-chromosome abnormalities (XXY, XYY, and a del(Y)(q12) with chromosome 15 satellites on Yq). Subtelomere-FISH and locus-specific FISH did not uncover any abnormality not known from the cytogenetic analysis. We also utilized a locally developed 35k cDNA microarray to investigate genomic copy-number changes in a subset of this group. To ensure the validity of the microarray results, we tested known chromosomal abnormalities found in our autistic individuals, including the 15q11.2q13 duplication and a terminal 2q37.1q37.3 deletion. The microarray results redefined the sizes of the duplication and deletion to 6.2Mb (from 13.1Mb) and 8.6Mb (from 12.2Mb) respectively. The 15q breakpoints reside in a proximal 36Kb region and distal 693Kb region while the terminal 2q37 breakpoints are in a 2.6kb region. The 35k microarray data confirmed the previous cytogenetic/FISH findings and was able to narrow abnormality breakpoints in a manner that would have been cumbersome with FISH techniques. Compared to commercially available array-CGH chips, the microarrays employed here were extremely cost-effective.

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Result of exploring pathogenesis of congenital disorders using array-CGH. *S. Hayashi[1,2,3], I. Issei[1,2], J. Inazawa[1,2].*
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PURPOSE: We have developed genomic microarray with 4523 BAC clones covering whole genome at intervals of about 0.7 Mb. Using the microarray we investigated aberration of copy numbers of genomic DNA to understand the pathogenesis of unknown congenital anomaly and/or mental retardation. METHOD: We have analyzed copy number aberrations in patients who were diagnosed known or unknown congenital disorder and/or whose phenotypes are not explained by conventional genomic analyze. More than 150 patients were analyzed. In each of them detected aberrations were confirmed by FISH. RESULT: Genomic aberrations were detected in about quarter of patients. They were deletion or duplication of chromosome materials, including interstitial or subtelomeric region, which size was 0.2 Kb at minimum. Some of them could explain entire phenotypes of patients. For example, it is thought that 4 Mb-wide deletion of 9p subtelomeric region which had not been detected by G-banding would cause mild phenotype of 9p monosomy syndrome. Given another example, we detected in a girl 2 Mb-wide interstitial duplication of Xp including a gene associated with maturation of ovary and it was thought that overexpression of the gene related to precocious puberty. Some of aberrations had possibilities to be large-scale copy number variation (LCV). Familial analyses were carried out, and aberrations also detected in related healthy people was thought not to be pathogenic. CONCLUSION: Array-CGH is thought to be significant technique to explore genomic aberrations. However LCV makes it to more difficult to evaluate whether the aberration was pathogenesis. Thus the construction of LCV database is important. Development of LCV database information about LCV will help us to know relationship between genomic aberrations and pathogenesis of genomic disorders as well as phylogenic study.

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Proximal 14q deletion overlaps with the HPE8 locus and is associated with corpus callosum agenesis. *G. Macintyre[1], K. Schlade-Bartusiak[1], L. Rodriguez[2], P.C. O'Brien[3], M.A. Ferguson-Smith[3], D.W. Cox[1].* 1) Dept Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Instituto de Salud Carlos III, Madrid, Spain; 3) Centre for Veterinary Science, University of Cambridge, Cambridge, United Kingdom.

Interstitial deletions and translocations of 14q11-12 have been associated with diseases including holoprosencephaly, schizophrenia (NPAS3), benign hereditary chorea (NKX2.1), tetramelic mirror image polydactyly (MIPOL1) and oligodontia (PAX9). We have characterized a *de novo* deletion in a female who presented in infancy with microcephaly, hypotonia and agenesis of the corpus callosum. At birth, weight was 50th percentile, length was 25th percentile and occipital-frontal circumference (OFC) was 25th percentile. At 2 years 2 months, weight, length and OFC were under 3rd percentile and generalized hypotonia, triangular facies, lack of speech and psychomotor delay were evident. Chromosomal analysis by G-banding revealed a karyotype initially interpreted as 46XX, del (14)(q11.2;q13.1). Parents were both 33 years old and of normal karyotype. FISH analysis with BAC genomic clones and STS marker analysis on flow sorted chromosomes were then used to ascertain the full extent of the deletion. The deletion was subsequently defined as del(14)(q12;q13.3), which spans at least 10Mb, and maps to a locus we have defined for holoprosencephaly (Kamnasaran et al., 2005). This region also contains a gene defective in two patients with schizophrenia (Kamnasaran et al., 2003). The proband has features in common with other cases with 14q deletions mapping to this region. The described deletion affects over 70 genes, including a number of genes expressed in the brain, such as FOXG1B and TITF1, two transcription factors required for normal forebrain development.

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Genome tilepath microarray analysis of copy number polymorphisms and methylation profiles in Autism. *S.G. Gregory[1], J. Connelly[1], R. Cote[1], C. Sheedy[1], M. Schemmel[1], J. Virgadamo[1], R. Abramson[3], H. Wright[3], R. Delong[2], M. Cuccaro[1], J.M. Vance[1], M. Pericak-Vance[1].* 1) Duke Center for Human Genetics, DUMC, Durham, NC; 2) Dept of Pediatric Neurology, DUMC, Durham, NC; 3) Dept of Neuropsychiatry, SOM-USC, Columbia, SC.

Autistic disorder (AutD) is a neurodevelopmental disorder characterized by disturbances in social, communicative, and behavioral functioning. There have been several linkage genetic screens and numerous association studies, however no gene has been identified. An alternative approach is the characterization of chromosomal rearrangements within individuals with AutD. It has been established that 5% and 48% of individuals with idiopathic and non-idiopathic autism, respectively, have chromosomal rearrangements. Additionally, AutD patients have been described with mutations in MECP that are known to be associated with abnormal methylation changes in the genome. This suggests that chromosome rearrangements or epigenetic changes in gene regulation could be responsible for AutD. Here we describe the novel use of high-resolution tilepath microarray (of chromosomes 1, 3, 4, 7, 10, 15, 17, 19, X, Y and 1Mb) to identify regions of chromosomal aberration using 42 AutD individuals, 9 maternal and 2 paternal DNAs. HrCGH analysis identified a minimal region of genomic amplification of 6Mb between clones RP11-467N20 and RP11-483E23 in 15q11.2 - 15q13.1 within 12 AutD individuals with isocentric chromosomes. The interval is bordered by two regions of normal copy number polymorphism, is covered by 50 tilepath clones (containing 4 genomic gaps) and encompasses 21 genes annotated within Ensembl. Methylation profiles were generated for 6 of the individuals for whom we generated hrCGH data. Using a recently published technique (Inazawa et al.) we have been able to identify increased methylation (>2 fold) of 25 clones in at least two or more of the 7 AutD cases. Genomic localization of clones exhibiting differential methylation places 7 of the 25 clones within known regions of AutD linkage, on chromosome 3q26, 7q32, 15q11-13, 16p13 and 17q12. This novel approach has great potential in helping elucidate the causal mechanism of AutD.

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Variation in IRF6 contributes to nonsyndromic cleft lip and palate. *S.H. Blanton[1], A. Cortez[2], S. Stal[3], J.B. Mulliken[4], R.H. Finnell[5], J.T. Hecht[2].* 1) Univ of Virginia Hlth Sci Ctr, Charlottesville; 2) Univ of Texas Medical School, Houston; 3) Texas Childrens Hospital, Houston; 4) Children's Hospital, Boston MA; 5) Institute for Biosciences and Technology, Texas A&M, Houston.

Cleft lip with or without cleft palate is a common birth defect found in more than 300 recognizable syndromes, but more often is observed as an isolated birth defect, called nonsyndromic cleft lip with or without cleft palate (NSCLP). NSCLP occurs in approximately 1/700 live births, causes significant facial anomalies and may result in significant morbidity, in addition to lifelong medical and social consequences. While there have been a number of attempts to identify the genes responsible for this disorder, the results have not been consistent among populations and no single gene has been identified as playing a major susceptibility role. Van der Woude syndrome, a disorder characterized by lip pits with or without cleft lip/palate, results from mutations in interferon regulatory factor 6 (IRF6) in many cases. Recently, Zucchero et al (2004) detected an association between SNPs in IRF6 and NSCLP in a number of different populations. A subsequent study by Scapoli et al (2005) confirmed this association in an Italian population. We examined the same four SNPs as Scapoli in our large, well characterized sample of NSCLP families and trios. The sample consists of 51 multiplex families, 184 simplex parent-child trios and 21 parent-child trios with a positive family history. While there was no evidence of linkage, either parametric or nonparametric, FBAT analysis of the individual SNPs in the sample detected an increase in the transmission of the C allele (major allele) (216 vs 201) ($p=0.05$) for rs2013162. In general, HBAT detected an increased transmission of haplotypes with this C allele matched by a decrease in the transmission of haplotypes with the A allele. The most significant results were obtained for the C-A-X-T haplotypes ($p=0.009$). Replication of positive findings in complex diseases has been difficult. That three different studies have yielded significant results for an association between IRF6 and NSCLP suggests that this is an important gene and clearly warrants further study.

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Molecular genetics approaches in human congenital diaphragmatic hernia. *S. Kantarci[1], B.R. Pober[1,2], M. Russell[1], D. Casavant[1], C. Lee[3], P.K. Donahoe[1].* 1) MassGeneral Hospital for Children,Boston, MA; 2) Childrens Hospital, Boston, MA; 3) Brigham & Womens Hospital,Boston, MA.

Congenital Diaphragmatic Hernia (CDH) is a relatively common developmental anomaly with a high mortality primarily due to lung hypoplasia and pulmonary hypertension. Little is known about the etiology of CDH. Just over half of patients have isolated CDH, while the remainder have associated birth defects, chromosomal abnormalities or single gene disorders. We aim to identify genes involved in CDH in our carefully phenotyped cohort of patients by application of several complementary strategies. We have sequenced 45 of the 152 enrolled patients for 21 candidate genes (ROBO1, ROBO2, SLIT2, SLIT3, FOX1, GATA4, etc.) identified from animal models, expression and functional patterns, or chromosomal hot spots. A web-based polymorphism phenotyping program, PolyPhen, was used on all nonsynonymous SNPS to predict the effect of amino acid changes on the structure and function of the protein. We found several potentially damaging SNPs not previously reported in the dbSNP homepage, nor in 970 chromosomes from an ethnically matched control group recruited as part of this study. However these SNPs were present in an unaffected parent. Functional studies of selected ROBO1 SNPs are underway and we also plan to expand the size of the control group. We are using 1Mb resolution array-based Comparative Genomic Hybridization (aCGH) to detect possible microdeletions or microduplications on 30 syndromic and nonsyndromic patients and detected two abnormalities involving apparently de novo deletions of chromosomes 16q12.2 and 1q42 respectively. Prior cases with deletions of 1q42 suggest this is a CDH hotspot. We are performing homozygosity mapping by application of the 10K Affymetrix chip to a large inbred kindred. This family contains several members affected with an autosomal recessive multiple anomaly condition which has CDH as a cardinal feature. These numerous strategies will identify genes, which when deleted or mutated, contribute to the development of CDH. These approaches provide a model for elucidating the genetic basis of common, but etiologically heterogeneous, birth defects.

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***AHSG* gene polymorphisms are associated with coronary artery calcification in the Diabetes Heart Study.** *K.P. Burdon, A.B. Lehtinen, J.P. Lewis, C.D. Langefeld, J.T. Ziegler, B.I. Freedman, D.W. Bowden.* Wake Forest Univ Sch Med, Winston-Salem, NC.

The 2-Heremans-Schmid glycoprotein (*AHSG* or fetuin-A) plays a major role in preventing ectopic vascular calcium by inhibiting calcium phosphate precipitation *in vitro*. *In vivo*, sequence variants in the *AHSG* gene are associated with reductions in circulating AHSG levels and increased vascular calcified plaque. This elevation in vascular calcium predicts cardiovascular disease (CVD) morbidity and mortality. The purpose of this study was to evaluate whether polymorphisms in the *AHSG* gene contribute to subclinical CVD in type 2 diabetic (T2DM) subjects. The Diabetes Heart Study (DHS) predominantly consists of European American affected sibling pairs who have been carefully phenotyped for clinical and subclinical measures of CVD. Using single base extension mass spectrometry, we genotyped 11 single nucleotide polymorphisms (SNPs) across the *AHSG* gene in 1,180 individuals from the DHS. Each SNP was evaluated for association with quantitative measures of subclinical CVD risk, including coronary artery calcified plaque (CAC), carotid artery calcified plaque (CarAC), and intimal-medial carotid wall thickness (IMT). Four SNPs were significantly associated with CAC in European American T2DM subjects (0.001P0.023) when adjusting for age, gender, smoking status, and use of lipid lowering medications. Haplotype analysis of the 11 SNPs identified two 4-SNP haplotypes encompassing exon 6, intron 6, and exon 7 that were associated with CAC when adjusting for age, gender, ethnicity, diabetes status, smoking status, and use of lipid lowering medications. The CGCC haplotype was significantly associated with increased CAC scores (P=0.030), while the TGGA haplotype was significantly associated with decreased CAC scores (P=0.012). However, only one of the SNPs in these haplotypes was individually associated with CAC. The results of this study suggest that polymorphisms in the *AHSG* gene are associated with CAC, consistent with the biological role of AHSG in vascular calcification. *AHSG* gene polymorphisms may impact CVD morbidity and mortality in diabetics.

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Systematic screening of susceptibility for intracranial aneurysms on chromosome 7q11 using sliding window analysis and permutation test. *H. Akagawa[1,2], H. Onda[2], H. Kasuya[2], M. Kubota[3], A. Hata[4], T. Hori[2], A. Tajima[1], I. Inoue[1].* 1) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Department of Neurosurgery, Tokyo Women'fs Medical University, Tokyo, Japan; 3) Department of Neurosurgery, Chiba University, Chiba, Japan; 4) Department of Public Health, Chiba University, Chiba, Japan.

Rupture of intracranial aneurysms (IA) resulted in subarachnoid hemorrhage with high morbidity and mortality, therefore IA represents one of the major public health concerns.'@A genome-wide linkage study of IA and detected significant linkage on 7q11 (MLS = 3.22 near *D7S2472*), accordingly we performed systematic single nucleotide polymorphism (SNP) fine-mapping at the 4.6Mb linkage region. 166 SNPs of 26 positional genes were detected and genotyped. The SNPs were initially tested for allelic association evaluated by permutation test, which adjusts the significance level to account for multiple comparisons of the data sets. Fifteen SNPs showed significant associations (permutation P 0.05), and 9 of them were clustered within a region of ~400 kb centromeric to *D7S2472*. The locus contains *ELN*, *LIMK1*, and *CYLN2*. Systematic haplotype analyses and LD mapping in the 4.6 Mb region were performed using sliding window method showing gene-specific LD blocks in the 4.6 Mb region. Haplotypes having significant associations (permutation P 0.05) were clustered within the locus containing *ELN*, *LIMK1*, and *CYLN2* confirming the results of single-SNP associations. When the significance level was set at permutation P value under 0.01, associations were confined to only one LD block for both single and global haplotype comparisons. The pinpointed LD block involved 3'f end of *ELN* and 5'f regulatory region of *LIMK1* and SNPs in the 3'funtranslated region (3'UTR) of *ELN* and the promoter region of *LIMK1* were significantly associated with IA. The functional impact and relationship of these variants were under investigation.

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Pharmacogenetic modulation of clopidogrel antiplatelet effects: role of sequence variations of the CYP3A4 gene.

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Clopidogrel is activated by cytochrome P450 (CYP) 3A4 and the activity of this enzyme contributes to variability in individual response to clopidogrel. Since metabolic activity of CYP3A4 is under genetic control, we hypothesized that gene sequence variations of CYP3A4 may modify individuals' response to clopidogrel. The CYP3A4*1B (-392A>G), CYP3A4*3 (M445T) and CYP3A4*16B (IVS10+12G>A) polymorphisms of the CYP3A4 gene were assessed in 127 patients (pts) undergoing coronary stent implantation: 45 receiving a 300 mg clopidogrel loading-dose (Group A) and 82 on long-term (>1 month) clopidogrel (75 mg/d) treatment (Group B). All patients were on aspirin (100 mg/d). Activated GPIIb/IIIa was assessed by whole blood flow cytometry in 2M ADP-stimulated platelets at baseline and 24 hours after loading-dose (Group A) and following long-term treatment (Group B). In Group A, clopidogrel response was defined according to the degree of inhibition of GPIIb/IIIa activation 24 hours after clopidogrel administration vs baseline values. Pts were classified as non responders, low responders, and responders when platelet inhibition was <10%, 10 to 29%, and 30%, respectively. Group B pts were divided in tertiles of platelet reactivity (low, moderate, and high). Only the IVS10+12G>A polymorphism was sufficiently polymorphic. G/A plus A/A pts were 9/45 (20%) of Group A, and 14/82 (17%) of Group B. In Group A, a higher number of non responders were observed among G/G pts (non responders: 36% vs 13%; low responders: 36% vs 0%; responders: 28% vs 87%; p=0.003). In Group B, G/G pts continued to have higher platelet reactivity and were less responsive to treatment according to tertile distribution (p=0.02). In conclusion, a polymorphism of the CYP3A4 gene seems to modulate clopidogrel antiplatelet effects in both the acute and chronic phases of treatment, and to contribute to individual variability in platelet responsiveness to clopidogrel.

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Innate Immunity Gene Polymorphisms in Regulation of CRP. *P. Kozlowski[1], D.T. Miller[1], D.I. Chasman[2], R. Lazarus[3], J. Suk Danik[2], R.Y.L. Zee[2], P.M. Ridker[2], D.J. Kwiatkowski[2].* 1) Division of Hematology; 2) Division of Preventive Medicine and Division of Cardiovascular Disease Prevention; 3) Channing Laboratory, Brigham and Womens Hospital, Boston, MA.

Atherosclerosis is a common and complex disease, and the leading cause of death and illness in developed countries. Its complex etiology includes both environmental and genetic components. In several large prospective studies, C-reactive protein (CRP) has been shown to be a marker of atherosclerotic cardiovascular disease risk, with effects that are independent of LDL-C and Framingham risk scores. We hypothesized that genetic variation with significant influence on CRP levels might be found in the genes of the innate immunity system. Therefore we performed a candidate gene association study examining common SNPs in 9 innate immunity genes (*CARD15*, *IRAK1*, *IRAK4*, *LBP*, *LY86*, *MEFV*, *TLR2*, *TLR4* and *NFKB1*) in relationship to CRP levels. 717 subjects from the Womens Health Study (WHS) population were selected: 359 samples with extremely low (<5th percentile; <0.2 mg/l) and 358 samples with extremely high CRP level (>86th percentile; >5 mg/l). We selected SNPs using publicly available resequencing data from three Programs for Genomic Applications (IIPGA, SeattleSNPs and NIEHS SNPs). Using a minor allele frequency threshold >5%; and linkage disequilibrium-based strategy (r2>0.8), we selected the 64 markers, which we genotyped by Sequenom, TaqMan and ARMS-PCR methods. In addition, we examined one non-synonymous SNP in *TLR4* and two non-synonymous SNPs in *CARD15* which were previously associated with atherosclerosis and Crohns Disease, respectively. We performed both univariate and haplotype analyses for all 67 SNPs in all 717 CRP discordant subjects. No significant association with CRP level was detected. In contrary to these results, a previous study using the same set of samples showed that SNPs in *CRP* gene are strongly associated with CRP level. Although the present study excludes a significant association of common SNPs in these innate immunity genes with CRP levels, it is possible that rarer alleles in these genes, or variation in other innate immunity genes, could be associated with variation in CRP.

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Composite genotypes of the IL-1 gene cluster predict IL-1 levels. *J. Rogus[1], J. Beck[2], K. Huttner[1], K. Kornman[1], S. Offenbacher[2].* 1) Interleukin Genetics, Waltham, MA; 2) Univ of North Carolina, Chapel Hill, NC.

Interleukin-1 (IL-1) is a potent cytokine involved in critical pathobiological processes of cardiovascular disease including recruitment of blood leukocytes, activation of downstream mediators such as IL-6 and CRP, and modulation of clot formation/dissolution. Extracellular release of IL-1 is regulated by complex feedback loops involving both the IL-1B gene, encoding the proinflammatory cytokine, as well as the IL-1RN gene encoding its natural antagonist. We evaluated the joint effect of two SNPs, one in the IL-1B promoter (IL-1B(-511) C>T) and one in the IL-1RN gene (IL-1RN(+2018) T>C), on IL-1 levels measured in non-invasive sampling of gingival crevicular fluid. For each SNP, genotypes were categorized as carriers (2/*) or non-carriers (1/1) of the minor allele. In 656 Caucasians, median IL-1 levels differed among the four resulting groups (p=.0003, Kruskal-Wallis). A similar pattern emerged in 185 African Americans, trending towards significance in this smaller cohort (p=.07, Kruskal-Wallis). We conclude that both IL-1B(-511) and IL-1RN(+2018) are associated with IL-1 levels in a manner best captured by the composite genotype patterns described. This insight can serve as a useful frame of reference for studies investigating the effect of the IL-1 gene cluster on clinical events such as myocardial infarction.

IL-1B	IL-1RN	n (Caucasian)	IL-1 (Caucasian)	n (African Am)	IL-1 (African Am)
1/1	1/1	216 (33%)	185.3	35 (19%)	94.6
1/1	2/*	87 (13%)	169.4	1 (<1%)	-
2/*	1/1	144 (22%)	152.6	124 (67%)	90.6
2/*	2/*	209 (32%)	137.9	25 (14%)	68.1

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Molecular analysis of impaired cholesterol efflux in subjects with familial low-HDL: The role of ABCA1. *J. Naukkarinen[1], A. Soro-Paavonen[2], M. Lee-Rueckert[3], A. Hiukka[2], H. Watanabe[2], S. Söderlund[2], M. Jauhiainen[1], P.T. Kovanen[3], L. Peltonen[1], M-R. Taskinen[2].* 1) Dept. of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; 3) Wihuri Research Institute, Helsinki, Finland.

The atherogenic effect of low-HDL is primarily due to impaired reverse cholesterol transport to the liver. Controversy prevails about the exact molecular pathogenesis of low-HDL, with most studies reporting an involvement of the ATP-binding cassette transporter 1 (ABCA1), critical for cholesterol efflux. We wanted to test the potential role of ABCA1 in well phenotyped Finnish low-HDL families, ascertained for population based age-sex specific 10th HDL percentile. We studied monocyte-derived macrophages isolated from both affected (HDL<10th percentile, n=22) and non-affected (n=7) men, as well as normolipidemic controls (n=21). SNP haplotypes and transcript levels of ABCA1 and the ability of ABCA1 to facilitate efflux of cholesterol to lipid-free ApoA-I from acetyl-LDL loaded macrophages in vitro were determined. Affected individuals showed altered carrier frequencies of the R219K allele, and in the whole study sample (n=73), a common three-SNP haplotype was associated with higher HDL-levels in a dose-dependent manner. The likelihood of an individual being affected when carrying either 0, 1 or 2 copies of this allelic haplotype were 80, 48 and 30%, respectively. Loaded macrophages from affected individuals demonstrated reduced cholesterol efflux, while exhibiting a significantly higher ABCA1 transcript level, that, however, dissipated after apoA-I facilitated cholesterol efflux. In summary, the carrier status for the common haplotype appears to have an effect on HDL levels, while it doesnt directly correlate with either the transcript level of ABCA1, or efflux efficiency. We infer that while part of the low HDL pathology may be explained by allelic variation of ABCA1, its higher expression in affected subjects may reflect a compensatory/rescue effort for a defect elsewhere in the complex pathway of cholesterol efflux and HDL formation.

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***LOXL2* polymorphisms are associated with susceptibility to intracranial aneurysms.** *B. Krischek[1,5], H. Yamada[1], H. Akagawa[1,2], H. Kasuya[2], H. Onda[2], M. Kubota[3], A. Hata[4], T. Hori[2], A. Tajima[1], I. Inoue[1].* 1) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Department of Neurosurgery, Tokyo Women'fs Medical University, Tokyo, Japan; 3) Department of Neurosurgery, Chiba University, Chiba, Japan; 4) Department of Public Health, Chiba University, Chiba, Japan; 5) Department of Neurosurgery, Philipps University Marburg, Marburg, Germany.

Background and Purpose- The etiology of intracranial aneurysms (IA) is complex involving both genetic and environmental factors. Four lysyl oxidase family genes (*LOXL1*, *LOXL2*, *LOXL3*, and *LOXL4*), catalyzing cross-linking of collagens and elastin that would enhance the strength of blood vessel wall, are considered to be plausible functional candidates for IA and were extensively screened for genetic susceptibility in Japanese and Korean IA patients.

Methods- Fifty-five single nucleotide polymorphisms (SNPs) of *LOXL1~4* were genotyped in 402 Japanese IA patients including 185 familial cases and 280 Japanese non-IA controls. Positively associated SNPs were further genotyped in 199 Korean patients and 194 Korean non-SAH controls. Allelic associations between the patients and controls were evaluated with ^{2} test or permutation test.

Results- In the first screening, two SNPs of *LOXL2* and two SNPs of *LOXL3* both locating next each other, showed significant association with IA (0.05). Then all of the IA patients of Japanese and Korean were genotyped. Significant allelic associations were observed with three synonymous SNPs located on exon 5 of *LOXL2* in comparison between Japanese patients and controls (Permutation P 0.05). The associations of these three SNPs were replicated in Korean group (Permutation P 0.05). The best evidence of association was observed in exon 5 [-28] GA SNP (Permutation P = 0.0024 in Japanese group, Permutation P = 0.011 in Korean group).

Conclusions- We identified the SNPs of *LOXL2* that would be a genetic risk to IA development in Japanese and Korean patients.

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Family based association analyses of the Histamine Receptor HRH1 gene in allergic disorders. *U. Christensen[1], T. Yndigegn[1], T.J. Corydon[1], A. Haagerup[1,2], H.G. Binderup[1], J. Vestbo[3,4], T.A. Kruse[5], A.D. Børglum[1].* 1) Institute of Human Genetics, University of Aarhus, Denmark; 2) Department of Paediatrics, Aarhus University Hospital, Skejby, Denmark; 3) Institute of Preventive Medicine, Kommunehospitalet, Copenhagen, Denmark; 4) North West Lung Centre, Wythenshawe Hospital, Manchester, UK; 5) Department og Clinical Biochemistry and Genetics, Odense University Hospital, University of Southern Denmark, Odense, Denmark.

Allergic diseases are a major global health problem with high and rising prevalence. The pathogenesis is multifactorial and has a substantial genetic part; for asthma, atopic dermatitis and hay fever the heritability is estimated to be 60-70%. We recently found linkage between the region 3p24-26 and atopic dermatitis. The gene coding for the histamine receptor H1 (HRH1) is located at 3p25 and is an obvious candidate gene owing to the central position of HRH1 as a key mediator of atopy and a regulator of Th1/Th2 balance. Here we present the results of a family based association study of two Danish samples comprising a total of 235 nuclear families with allergic diseases. We found significant association between 2 single nucleotide polymorphisms (SNPs) in the HRH1 promoter and several atopic phenotypes (e.g. positive skin prick test: p= 0.002). Haplotype analyses also revealed association with p-values as low as 0.0009 for atopic dermatitis with positive rast test. In silico analyses of these variations showed influence on the binding potential of conspicuous activation factors. Furthermore, a cell based reporter gene assay revealed that the -89A>G promoter variation radically reduced the transcription of the HRH1 gene.

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Design of a National Resource for Gene Discovery in Parkinsons Disease. *D.J. Horsford[1], J. Keen[2], J. Beck[2], D. Murphy[1], Q. Scudder[1], C. Royds[2], K. Gwinn-Hardy[1], S. Rich[3].* 1) NINDS, Bethesda, MD; 2) Coriell Cell Repositories, Camden, NJ; 3) Bowman Wake Forest University School of Medicine, Winston-Salem, NC.

The National Institute of Neurological Disorders and Stroke (NINDS) Repository at Coriell (http://locus.umdnj.edu/ninds/) is a public resource banking cell lines and DNA with clinical data in order to identify genetic risk factors in Parkinsons disease (PD) and other neurological disorders. To facilitate this, the NINDS sample collection is publicly available to both academic and industry sponsored investigators. The NINDS repository is designed to maximize gene discovery in neurological diseases by providing 1) high quality DNA from immortalized cell lines 2) detailed clinical data on both cases and controls and 3) power to detect the moderate effects of a single susceptibility gene by banking a large sample number. We hope to identify SNPs with approximately a 1.5-fold or greater relative risk. For common SNPs (with minor allele frequency >10%), this translates into an allele frequency difference of ~10% or more. With 5000 cases in a given disease, the expected allele frequency differences between cases (and controls) for a marker that accounts for 2% of the phenotypic variance ranges from 10% to 15%. Substantial statistical genetic and empirical data suggest that 5000 PD cases would be relevant for gene discovery of individual gene effects underlying risk of complex human disease. Should there be gene-gene or gene-environment interactions, the sample size required to maintain power would be increased by at least 4-fold. Thus, there is a clear rationale for continuing sample collection even once the current target of 5000 has been reached. We currently have publicly available 827 well-characterized PD subjects and 492 matched control subjects, of 2372 PD and 1217 controls collected and banked. Secondary validation of risk factor genes will also be possible in this collection, because of the availability of populations different from the original cohort which can be used.

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Genomic analyses and allele specific mRNA analysis using quantitative PCR. *A. Torinsson Naluai[1,2], C. Ladenvall[2], J. Danielsson[2], T. Martinsson[1,2], C. Jern[1].* 1) Göteborg Genomics, Core facilities at the Sahlgrenska Academy, Göteborg, Sweden; 2) Department of Clinical Genetics, Göteborg University, Sweden.

The SWEGENE Göteborg Genomics core facility carries out specific analyses using high throughput techniques. We give support in the experimental design for dissection of the genetic complexity of polygenic and monogenic diseases. The different methods available are DNA sequencing, genotyping of single nucleotide polymorphisms (SNPs) as well as microsatellites and quantitative PCR using the TaqMan chemistry. In the process of analyzing data we continuously work on developing new methods and applications.

We are presently developing a method using quantitative PCR for measuring different mRNA levels of heterozygote individuals.

It is well established that single nucleotide polymorphisms can influence the amount of mRNA produced. The method currently being developed provides a means to investigate allele-specific expression in vivo, using DNA from the same individual as an internal control. Differences in cDNA levels between alleles can be detected using quantitative PCR. If the SNP is somehow involved in regulation of mRNA expression or stability, and if no imprinting or external regulatory elements are involved, one of the alleles would express more mRNA than the other allele.

This technique has great potential in discriminating between possibly functional SNPs and non-functional SNPs on a transcriptional level.

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The genetics of type 1 diabetes complications: results from the National Disease Resource Interchange (NDRI) database. *M.C. Monti[1,3], E. Schlag[2], J.T. Lonsdale[2], L. Ducat[2], C. Montomoli[3], D.A. Greenberg[1].* 1) Div Stat Genetics, Dept. Biostat, Columbia Univ, NY NY; 2) National Disease Resource Interchange, Philadelphia PA; 3) Dept. Applied Health Sciences, Univ of Pavia, Italy.

Retinopathy, nephropathy and neuropathy are major complications of type 1 diabetes (T1D). Retinopathy and nephropathy show familial clustering, suggesting genes influence risk. Only a subset of diabetic patients have specific complications; thus, factors for complications may differ from those for diabetes risk. We analyzed a large cohort of T1D families to see if family history is a risk factor for retinopathy and retinopathy type, nephropathy, and neuropathy. Data: 6687 families (81,771 individuals) ascertained through at least 1 T1D offspring (index case). 5831 offspring and 694 parents had T1D; 742 parents had type 2 diabetes (T2D); 889 families were multiplex (source: NDRI database). Data on complications came from self-report questionnaires and medical records. We assessed retinopathy type using ADA guidelines. We used a case-control design nested on the cohort and logistic regression models that included potential confounding factors. We used families with exactly two, or exactly three T1D-affected offspring. Cases and controls were age-frequency matched. (Controls were T1D patients without the complication.) Our four major findings: 1) If a complication was present in a proband, that complication was significantly more likely to be found in T1D affected sibs ($p<.0001$ for all three complications). 2) If a parent had T2D, there was a significantly higher risk for retinopathy in offspring than without a T2D parent ($p=.048$). 3) If an index case has proliferative retinopathy, that form of retinopathy is more likely to appear in T1D affected sibs. 4) The presence of retinopathy increases the risk for other complications ($p=.002$), especially in T1D females. Our study shows that familial clustering exists for diabetic complications and the severity and progression of retinopathy tend to be familial. This is the largest cohort study to date of the complications of T1D and it confirms a strong familial, and likely genetically-based, risk for the devastating complications of T1D.

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12-lipoxygenase non-synonymous coding variants predict albuminuria in type 2 diabetes (T2DM): The Diabetes Heart Study (DHS). *Y. Liu[1], B.I. Freedman[1], K.P. Burdon[1], C.D. Langefeld[1], T. Howard[1], D.W. Bowden[1], L.E. Wagenknecht[1], C.C. Hedrick[2], S.S. Rich[1].* 1) Public Health Science/internal Medicine/Center for Human Genomics, Wake Forest University , Winston-Salem, NC; 2) Department of Medicine and the Cardiovascular Research Center, University of Virginia, Charlottesville, VA.

Hyperglycemia induces 12-lipoxygenase (12LO) and its arachidonic acid-derived products in endothelial and mesangial cells. This process can lead to endothelial dysfunction with cell hypertrophy and proliferation and result in diabetic nephropathy. We tested whether genetic variants in the 12LO gene (ALOX12) contributed to the development of albuminuria and whether this effect could interact with the hyperglycemic environments. Three ALOX12 haplotype-tagging SNPs (rs2292350, rs1126667 [R261Q], and rs2271316) were genotyped and urinary albumin:creatinine ratio (ACR) measured in 978 European American siblings (83% with T2DM) from 369 DHS families. Participants were categorized as: non-diabetic (non-T2DM), diabetic with hemoglobin A1c < 7% (controlled T2DM), and diabetic with hemoglobin A1c > 7% (uncontrolled T2DM). Tests of association were based on generalized estimating equations. The median ACR was 11.9 mg/g (interquartile range, 5.6-39.1). In the entire DHS sample, the overall test of the R261Q SNP genotypic association with ACR was significant (p =0.009). Compared to the 261Arg (R261Q) allele carriers, mean ACR adjusted for age, sex, smoking, and diabetic status was 42% higher among the 189 carriers of two 261Glu alleles (95% confidence interval, 10% to 83%; p=0.007). This association was significantly enhanced in the uncontrolled T2DM group, who had the highest ACR values (p for interaction across the 3 groups = 0.01). Further adjustment for other determinants of ACR or haplotype analysis revealed similar results. The 261Arg (R261Q) variant of ALOX12 is associated with greater degrees of albuminuria (ACR), predominantly among those with poorly controlled T2DM. Consistent with animal and cellular studies, these results provide further evidence of the importance of the 12LO pathway in the pathogenesis of human diabetic nephropathy.

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Sequence Variants and Haplotypes of the DUSP12/ATF6 Region on Chromosome 1q21 Are Associated with Type 2 Diabetes. *W.S. Chu[1], S.K. Das[1], X. Wang[1,2], R.L. Craig[1], S.C. Elbein[1,2].* 1) Medicine, University of Arkanasas for Medical Sciences, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR.

Linkage of type 2 diabetes (T2DM) to chromosome 1q21-q23 is well replicated across populations. We initially placed a 50 kb SNP map across the linked region in unrelated Caucasian individuals. We identified a strong association with marker rs1503814. We subsequently mapped the association to a highly conserved region just telomeric to this marker and extending through the gene DUSP12,10 kb downstream. Sequence analysis and typing of additional public SNPs narrowed the association to a region of 62.4 kb encompassing 10 kb upstream of DUSP12, the DUSP12 gene, and the 5 end of the ATF6 gene. We evaluated 61 variants, including 2 microsatellites, 2 insertion deletion variants, and 57 single nucleotide polymorphisms (SNPs) in Caucasians. No coding variants could explain the association. The associated SNPs were present in 3 LD blocks, the first including the index SNP, the second extending from 8.8 kb upstream to 10.3 kb downstream of DUSP12, and the third beginning just upstream of ATF6 and extending into intron 4. No single haplotype from these blocks was more strongly associated than individual variants. Of the 25 SNPs in Block 2, 15 were associated with T2DM ($p=0.0008$ to 0.042) , 2 showed a trend to association ($p<0.01$), and 5 were infrequent (MAF<0.1). Hence, only 2 SNPs in DUSP12 were both common and not associated with T2DM. Analysis of Block 2 SNPs in African Americans also showed an association of the microsatellite variant in the conserved nongenic region 8 kb upstream, but not other SNPs in this block. However, block 2 extended only 5 kb downstream in African Americans and did not encompass a second associated SNP 7.5 kb downstream from the ATG start site. We propose that SNPs and microsatellite variants in 3 blocks provide independent contributions to the association with T2DM in both Caucasians and African Americans. Both DUSP12, which regulates glucokinase activity in liver and likely -cell, and ATF6, an endoplasmic reticulum stress protein, are strong functional candidates for T2DM.

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Admixture-adjusted association between the estrogen receptor alpha gene and type 2 diabetes in an African American population. *K.L. Keene[1], C.J. Gallagher[1,2], J.C. Mychaleckyj[1,3], C.J. Gordon[1], C.D. Langefeld[4], B.I. Freedman[3], D.W. Bowden[1,2], M.M. Sale[1,3].* 1) Center for Human Genomics; 2) Department of Biochemistry; 3) Department of Internal Medicine; 4) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

Evidence for association with type 2 diabetes mellitus (T2DM) has been observed in a 41 kb region spanning intron 1-intron 2 of the estrogen receptor alpha gene (ESR1) in our African American case-control population sample. Two single nucleotide polymorphisms (SNPs) in intron 2 (rs11155818 P=0.00004; and rs1033182 P=0.020) as well as three haplotype blocks of high linkage disequilibrium (LD) spanning this region (global P<0.001) were significantly associated with T2DM. The objective of this study was to adjust association analyses for the contribution of admixture. Twenty-three ancestry informative markers (AIMs) and 17 SNPs in the associated region of the ESR1 gene were genotyped in 282 non-diabetic Caucasian and 120 non-diabetic African individuals, as well as 380 African American T2DM cases and 271 African American population controls. Using ADMIXMAP, the estimate for the mean proportion of African ancestry was 0.804 (95% credible interval: 0.779-0.831) in cases, and 0.815 (0.789-0.840) in controls. SNPs rs11155818 and rs1033182 remained significantly associated with T2DM after adjusting for admixture. The allelic association p-values, based on 20,000 iterations, changed from 0.00004 (unadjusted) to 0.00016 for rs11155818, and 0.020 (unadjusted) to 0.022 for rs1033182. Haplotype analysis also indicated that the three haplotype blocks were still significantly associated with T2DM (P<0.001) after adjusting for admixture, and all 7 significantly associated individual haplotypes (P<0.05) within the three blocks remained significant at or below this level. These analyses suggest that admixture does not account for the observed single SNP and haplotype association results between this region of ESR1 and T2DM in our African American population sample.

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Association of the proprotein convertase subtilisin/kexin-type 2 (PCSK2) gene with type 2 diabetes in an African American population. *T.S. Leak[1], C.D. Langefeld[2], C.J. Gallagher[1,3], J.C. Mychaleckyj[1,4], B.I. Freedman[4], D.W. Bowden[1,3], M.M. Sale[1,4].* 1) Center for Human Genomics; 2) Department of Public Health Sciences; 3) Department of Biochemistry; 4) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

The proprotein convertase subtilisin/kexin-type 2 (PCSK2) gene, located on chromosome 20p11.2, is a strong functional candidate for type 2 diabetes (T2DM). PCSK2 cleaves the proinsulin molecule on the COOH-terminal side of Lys64-Arg65, which joins the C-peptide and the A-chain domains, and proinsulin levels have been reported as a predictor of T2DM. Additionally, a genome wide scan performed in 638 African American affected sibling pairs (ASP) from 247 families revealed modest evidence for linkage to T2DM age at diagnosis at 39cM on chromosome 20p near microsatellite D20S470 (LOD 1.61; p=0.0075). Ordered subsets analysis also provided evidence for linkage to a T2DM subset with later age at diagnosis (max. LOD 2.50, p=0.027). The PCSK2 gene is within the LOD-1 interval of both linkage peaks. We genotyped 29 single nucleotide polymorphisms (SNPs) across this gene in 380 unrelated African American individuals with T2DM and end-stage renal disease (T2DM-ESRD) and 278 African American population controls without a known diabetes diagnosis. Four SNPs were found to be associated with T2DM: rs4814597 (p=0.047), rs1609659 (p=0.041), rs2021785 (p<0.001) and rs2269023 (p=0.040). Analysis with Haploview, extending the block if pairwise D>0.7, revealed two blocks of high linkage disequilibrium (LD) containing 5 SNPs and 7 SNPs. Haplotype analyses using Dandelion showed the 7-SNP haplotype in the distal region of the gene was significantly associated with T2DM, with an overall haplotype p-value of 0.008. Within this block, there was a significant risk haplotype (6.8% in cases, 2.8% in controls; OR 2.46, p=0.022) counterbalanced by a protective haplotype (14.6% in cases, 22.9% in controls; OR 0.58, p=0.0066); rs2021785 distinguishes between these two haplotypes. The PCKS2 gene appears to play a role in susceptibility to T2DM in the African American population.

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Re-sequencing and Functional Testing of PTPN1 Haplotypes Associated with Type 2 Diabetes and Insulin Sensitivity in Caucasian and Hispanic Americans. *N.D. Palmer, J.C. Mychaleckyj, G.A. Hawkins, D.W. Bowden.*
Wake Forest Univeristy, Winston-Salem, NC.

Protein-tyrosine phospatase-1B (PTP-1B), encoded by the PTPN1 gene, catalyzes the dephosphorylation of proteins at tyrosyl residues. PTP-1B negatively regulates insulin signaling by dephosphorylating the insulin receptor. In four independent populations, we observed significant evidence of association between type 2 diabetes (T2DM) and quantitative measures of glucose homeostasis and SNPs contained in a single LD block spanning ~110kb, encompassing PTPN1. Haplotype analysis suggests the presence of two common haplotypes: a T2DM risk haplotype (36%) that is associated with reduced insulin sensitivity and a second common haplotype (43%) which appears to be neutral in genetic epidemiological modeling. Alternate models of risk association are possible however, including the presence of multiple uncommon PTPN1 risk variants contributing to overall observation of T2DM risk. In order to identify a causal variant(s), we have carried out detailed re-sequencing of this LD block in 24 Caucasian and 24 Hispanic Americans. The DNA sequence from the 110,037bp region was determined from 220 overlapping fragments amplified from subject DNAs. We have identified 206 polymorphisms: 123 reported in dbSNP and 83 newly identified. Of the newly identified SNPs, 19 are insertion/deletion mutations and include 2 mini- and 2 microsatellites. An additional 208 reported polymorphic sequences were not found. This re-sequencing effort provides a comprehensive collection of the DNA sequence variation of the PTPN1 gene. In an effort to assess whether PTPN1 haplotypes are associated with altered levels of mRNA expression, a total of 36 B-lymphocyte cell lines were obtained from Caucasian individuals with known genotypes. mRNA was extracted from the cells and quantitated by real-time RT-PCR. There was no statistical difference in expression levels based on genotype in these cell lines. However, these cell types are not ideal models for regulation of glucose homeostasis and parallel experiments are underway in cultured primary myocytes from skeletal muscle biopsies of patients with known PTPN1 genotypes.

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Type 2 diabetes (T2D) and quantitative trait (QT) association analysis of MODY genes in Finns. *L.L. Bonnycastle[1], C.J. Willer[2], C.P. Burrill[1], A.U. Jackson[2], R.M. Watanabe[3], L.J. Scott[2], P.S. Chines[1], K.N. Conneely[2], N. Narisu[1], M.R. Erdos[1], A.J. Swift[1], N.L. Riebow[1], S.T. Enloe[1], T.A. Buchanan[3], T.T. Valle[4], J. Tuomilehto[4], R.N. Bergman[3], K.L. Mohlke[5], M. Boehnke[2], F.S. Collins[1].* 1) NHGRI, NIH, Bethesda, MD; 2) U. Michigan, Ann Arbor, MI; 3) U. Southern California, Los Angeles, CA; 4) Nat'l Public Health Inst., Helsinki, Finland; 5) U. North Carolina, Chapel Hill, NC.

T2D is a multifactorial polygenic metabolic disorder characterized by defects in both insulin action and secretion. In contrast, maturity-onset diabetes of the young (MODY), an autosomal dominant form of diabetes, is primarily due to impaired insulin secretion as a result of variations in one of at least 6 genes: *HNF4A*, *GCK*, *TCF1*, *IPF1*, *TCF2* and *NEUROD1*. Several of the MODY genes are part of a transcription factor network regulating gene expression in the pancreas and liver. Reports of T2D association with variants in *HNF4A*, *GCK*, *TCF1* and *IPF1* support the hypothesis that subtle genetic variants in MODY genes may confer susceptibility to T2D. To complement our ongoing studies of *HNF4A*, we have undertaken a comprehensive gene-based association study to determine the relevance of 5 other MODY genes in T2D in Finns. For each gene and its flanking regions (20 kb 5 and 5 kb 3), we selected non-redundant SNPs (r^2<0.8 with other SNPs) either from the HapMap database (www.hapmap.org) or a private LD map source (D. Altshuler, personal communication). Thus far we have tested 77 SNPs on 795 index cases from T2D families, and 655 normal glucose-tolerant controls. We performed permutation tests to derive empirical p-values that account for multiple testing within each SNP. Associations with *NEUROD1*, *TCF1* and *TCF2* have been identified, with p-values of 0.0007, 0.02, and 0.01 respectively. We also examined whether MODY gene SNPs were associated with diabetes-related QTs. For 6 SNPs in 3 genes (*IPF1*, *GCK* and *TCF2*), we found significant ($p< 0.05$) trait associations in the glucose-tolerant individuals. One SNP in *TCF2* exhibited association with both T2D and QTs. Our data suggest that one or more of these MODY genes may also play a role in T2D pathogenesis.

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A Genetic Variant in the Neuropeptide Y Gene (NPY) is Associated with Energy Balance and Obesity in Pima Indians. *Y.L. Muller, C. Bogardus, L. Baier.* Diabetes Molecular Genetics, NIDDK, NIH, Phoenix, AZ.

Neuropeptide Y (NPY) is a peptide encoded by the NPY gene on human chromosome 7q15. This peptide affects multiple metabolic processes, including regulation of energy balance, insulin release, and lipoprotein lipase activity, and administration of NPY in the central nervous system of rat has been shown to be a powerful stimulant of feeding behavior. Therefore, NPY was analyzed as a candidate gene for obesity in Pima Indians. The coding region and ~2 kb of the promoter region of the NPY gene was sequenced in 96 non-first degree related extremely obese Pima Indians (body mass index [BMI]=50.5-79.6kg/m2). Seven single nucleotide polymorphisms (SNP) were identified, including two synonymous SNPs (Ser50Ser, Ser68Ser) and five promoter SNPs. These variants were genotyped for a family-based association analysis in 1037 Pima Indians. The Ser50Ser (A/G), with a minor allele frequency of 0.07 (G), was associated with BMI (p=0.03 under an additive model, p=0.05 under a recessive [A/A vs. A/G+G/G] model, adjusted for age, sex, birth-year and Pima heritage). Among 252 non-diabetic, full-heritage Pima subjects who had undergone detailed metabolic testing, subjects with the A/G genotype (n=31) for Ser50Ser had a lower plasma glucose level at 30 min and 60 min during an oral glucose tolerant test compared to subjects with the A/A genotype (n=221) (1393 vs. 1482mg/dl, p=0.007; 1435 vs. 1522mg/dl, p=0.04, respectively, adjusted for age, sex, % fat and family membership). This SNP was additionally associated with 24-hour energy intake and energy balance in 176 non-diabetic, full-heritage Pima subjects, as measured in a respiratory chamber. Subjects with the A/G genotype (n=23) had a lower energy intake and were in more negative energy balance as compared to subjects with the A/A genotype (n=153) (223568 vs. 234229kcal/day, p=0.003; -110 48 vs. -5113kcal/day, p=0.04, respectively, adjusted for age, sex, % fat and family membership). These data indicate that a variant in the NPY gene may influence susceptibility to obesity by influencing energy balance.

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Polymorphisms in the FAS and FASL genes in Type 2 diabetes and in phenotypic traits caused by demise in beta cell mass and function. *R. Nolsoe[1], Y.H. Hamid[1], F. Pociot[1], S. Paulsen[1], K.M. Andersen[1], K. Borch-Johnsen[1], T. Drivsholm[2], T. Hansen[1], O.B. Pedersen[1,3], T. Mandrup-Poulsen[1,4].* 1) Steno Diabetes Ctr, Gentofte, Denmark; 2) Research Centre for Prevention and Health, Copenhagen County, Glostrup University Hospital, Denmark; 3) Faculty of Health Science, University of Aarhus, Denmark; 4) Department of Molecular Medicine, The Rolf Luft Center for Diabetes Research, Karolinska Institute, Stockholm, Sweden.

Aims/hypothesis. Type 2 diabetes is caused by a failure of the pancreatic beta cells to compensate for insulin resistance leading to hyperglycemia. There is evidence for a central role of a decline in beta cell mass due to increased beta cell apoptosis in the pathophysiology of Type 2 diabetes. Elevated glucose concentration upregulates Fas in human beta cells and induces apoptosis due to constitutive expression of FasL. The aims of this study were to test if polymorphisms in the FAS and FASL genes were associated with Type 2 diabetes or with estimates of pancreatic beta cell function and mass in normal-glucose tolerant (NGT) Caucasians. Methods. We genotyped two functional promoter variants; FAS-670 G/A and FASL-844C/T as well as a microsatellite in 3'UTR of FASL in 549 Type 2 diabetic patients and 525 normal-glucose tolerant subjects. Fishers exact test was applied to examine differences in allele frequencies and genotype distributions between Type 2 diabetic and NGT subjects. Phenotypic differences between the genotype groups among NGT subjects were tested with a general linear model including gender and genotype as fixed factors and age and BMI as covariate factors. Results. We found a significant association to Type 2 diabetes for the most frequent allele (16-repeat) of the FASL microsatellite (p value 0.04).There were no significant difference in genotype distribution between type 2 diabetic patients and normal-glucose tolerant subjects for the FAS-670G/A and FASL-844C/T polymorphisms (p values 0.16 and 0.4) or in the allele distribution for these two polymorphisms (p values 0.3 and 0.4). We found no overall significant association of any of the phenotypic traits to any of the polymorphisms tested in the FAS and FASL genes. Conclusion. We conclude that there is an association of the 16-repeat allele of the FASL microsatelitte to Type 2 diabetes in Danish Caucasian subjects.

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Association of genetic variants in *VLDLR* with Type 2 Diabetes Related Traits in Mexican Americans. *V.S. Farook[1], S. Puppala[1], R. Arya[2], J. Schneider[1], S. Fowler[2], T.D. Dyer[1], S.A. Cole[1], L. Almasy[1], J. Blangero[1], M.P. Stern[2], R. Duggirala[1].* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) University of Texas Health Science Center, San Antonio, TX.

The incidence of metabolic syndrome (MS), a predictor of type 2 diabetes (T2DM) and coronary heart disease, continues to increase worldwide. Linkage between a broad region on chromosome 9 and susceptibility to T2DM-MS related traits has been established in several family studies including ours. One of the positional candidate genes in this region is *VLDLR* (very low density lipoprotein receptor) which plays an important role in triglyceride and cholesterol metabolism. To assess if variants in *VLDLR* may contribute to T2DM-MS related traits, we chose 9 single nucleotide polymorphisms (SNPs) from the public databases and genotyped ~720 individuals who participated in the San Antonio Family Diabetes/Gallbladder Study. All SNPs are in HW equilibrium. We performed association analyses on these SNPs for 10 diabetes related phenotypes using measured genotype analysis [MGA], quantitative transmission disequilibrium test [QTDT], and quantitative trait linkage disequilibrium [QTLD] test within the variance components analytical framework (SOLAR). All techniques yielded similar results; only QTLD results (p 0.05) and, in the case of population stratification QTDT results are reported. Of the 9 SNPs studied, 3 (rs10967213; rs2290465; rs8210) showed significant association with insulin (p = 0.028-0.043), 2 (rs1551411; rs2290465) with MS (p = 0.025 and 0.010) and 1 (rs8210) with HDL-cholesterol (p = 0.012). Interestingly, one of the SNPs (rs10967213) showed strong associations with 6 phenotypes: adiponectin (p = 0.004), insulin (p = 0.028), leptin (p = 0.002), triglycerides (p = 0.033), BMI (p = 0.005), and waist circumference (p = 0.011); and, a moderate association with MS (p = 0.066). Although, an association between T2DM and a variant in *VLDLR* has been reported in a Japanese population, we show here for the first time a variant in *VLDLR* to be associated with several metabolic traits. In conclusion, variants in *VLDLR* are significantly associated with T2DM-MS related phenotypes in Mexican Americans.

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Association Between Sequence Variants in the Positional Candidate Gene *GNAT3* Near *CD36* Region and Metabolic Syndrome in Mexican Americans: Preliminary Findings. *S. Puppala[1], V.S. Farook[1], R. Arya[2], J. Schneider[1], S. Fowler[2], T.D. Dyer[1], S.A. Cole[1], L. Almasy[1], J. Blangero[1], R. Duggirala[1].* 1) Southwest Fndn for Biomed Res, San Antonio, TX; 2) Univ of Texas Health Sci Ctr, San Antonio, TX.

We identified a major gene for Metabolic Syndrome (MS, NCEP/ATPIII) on chromosome 7q21 between the markers D7S2212 and D7S821 in Mexican Americans, previously. Other studies also implicated a broad region on chromosome 7q to influence MS related phenotypes. One of the important positional candidate genes in the 7q21 chromosomal region that has functional relevance to MS is *CD36* or *FAT* (fatty acid translocase). We have started exploring several genes in or around *CD36* gene region that are within the 1 LOD-unit support interval of our major linkage peak on 7q21. In the present study, we have screened the *GNAT3* (Guanine nucleotide binding protein, alpha transducing 3) gene, which is located upstream of *CD36* to identify the sequence variants that may explain our initial MS linkage findings in Mexican Americans. We obtained SNP information from public databases, and typed 5 SNPs (rs799975, rs6942728, rs1473122, rs11760281, and rs10234980) from the *GNAT3* region using data from the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) (N = ~720). We performed association analyses between MS and the 5 SNPs, using the measured genotype analysis within the variance components (threshold model) framework (SOLAR). All 5 SNPs are in HWE. The minor allele frequencies ranged from 3% to 40%. Of the SNPs examined in the *GNAT3*, 4 SNPs showed significant or suggestive association with MS after adjusting for significant age effects: rs799975 ($p = 0.058$), rs6942728 ($p = 0.088$), rs1473122 ($p = 0.028$), and rs11760281 ($p = 0.0006$). Given evidence for association of variants in the *CD36* region with MS related phenotypes in other studies, it is interesting to find a highly significant association between MS and rs11760281 (minor allele frequency = 40%). In summary, our preliminary data signify the important association of variants in the *GNAT3* with MS in Mexican Americans, and the regions in or around *CD36* are being investigated further.

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Association of *ACDC, PPARG* and *LEP* gene polymorphisms in type 2 diabetes and obesity among the Samoans.

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Type 2 diabetes (T2DM) and obesity have reached epidemic proportions on a global scale. Both phenotypes result from a complex interaction of genetic and environmental factors. We have tested for association of variants in three plausible candidate genes (*ACDC*, *PPARG*, *LEP*) with T2DM and obesity among the Samoans of Polynesia, who have a very high prevalence of both diseases. We analyzed eight Single Nucleotide Polymorphisms (SNPs) in *ACDC* encoding for adiponectin, which is expressed in adipose tissue and is correlated with the severity of insulin resistance; seven SNPs in *PPARG*, which regulates adipocyte differentiation, lipid metabolism and insulin sensitivity; and six SNPs in *LEP* encoding for leptin known for its role in body weight regulation. For T2DM, we recruited 166 cases and 216 controls; and for obesity, we chose phenotypically extreme obese (mean BMI = 44.04, N = 96) and lean (mean BMI = 23.73, N = 87) subjects. To evaluate for any possible population stratification, we genotyped 30 null SNPs spanning the genome in all our samples. A -value of 0.97 was obtained negating any spurious association from population substructure. In *ACDC*, we observed significant association at the haplotype level ($P = 0.014$) with T2DM. No significant association was observed either at genotypic or allelic level with T2DM and obesity. In *PPARG*, rs10510411 showed association ($P = 0.039$) with obesity, and also significant association was observed at the haplotype level ($P = 0.006$) with T2DM. In *LEP*, association at haplotype level was observed ($P = 0.039$) in the diabetes group although none was observed at genotypic or allelic level with T2DM and obesity. Our study demonstrates a possible role of variants in *ACDC*, *PPARG* and *LEP* in the pathogenesis of T2DM and obesity related phenotypes among the Samoans. Supported by grants DK59642, DK55406.

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Association of *CAPN10* Haplotypes with Type 2 Diabetes in the Saudi Population. *K. Al-Rubeaan[1], F. Imtiaz[2], O. Alsmadi[2], S.M. Wakil[2], P. Carroll[2], M. Rajab[2], S. Al-Katari[1], M. Al-Katari[1], B.F Meyer[2].* 1) Diabetes Center, King Abdulaziz University, Riyadh, Saudi Arabia; 2) Aragene, KFSH, Riyadh, Saudi Arabia.

The biological function(s) of CAPN10 is/are unknown. One role for CAPN10 is believed to be triggering of insulin exocytosis from beta cells. Variations in CAPN10 were found to associate with type 2 diabetes (T2D) in various populations. Inheritance of a specific haplotype combination defined by three single-nucleotide polymorphisms (SNP 43, 19, and 63) was found to associate with a threefold increased T2D risk. SNP 43, 19, and 63 are in non-coding regions of CAPN10 and are believed to affect the transcriptional regulation of CAPN10. To date there has been no reported studies of the association of CAPN10 haplotypes with T2D in Arab populations. We used fragment analysis and sequencing for genotyping of SNP 19 and SNPs 43/63 respectively. Alleles for SNPs 43, 19 and 63 are G/A, 2/3-32bp repeats, and C/T respectively. SNP alleles were assigned either 1 or 2 in the order mentioned for each pair. 480 patients diagnosed with T2D according to WHO criteria were studied along with a normal control set (age >60 with fasting blood glucose <7mM). Haplotypes (SNP 43, 19, 63) were ascertained where no ambiguity was present or deduced on a most likely basis. Frequencies of the eight possible haplotypes were identified and haplotype combinations assigned for each patient. 111/111 was the most common combination seen in both patients and controls. Previous studies indicated the 112/121-haplotype combination to be most strongly associated with T2D in several populations. However, this is not the case in the Saudi population where the 111/121-haplotype combination was significantly increased in T2D patients compared with normal controls (p=0.006). SNP-44 (C/T) of CAPN10 was also typed for all patients/controls and similarly to results seen in UK T2D patients, the C allele was shown to be associated in our population (p=0.019). In addition, frequencies of various other haplotypes and their combinations in the Saudi T2D patients are significantly different to those reported in other populations.

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A functional NFB1 promoter polymorphism and type I diabetes in Norwegian trio families. *M.C. Eike[1], G. Joner[2], K. Dahl-Jørgensen[2], K.S. Rønningen[3], D.E. Undlien[4], E. Thorsby[1], B.A. Lie[5].* 1) Inst. of Immunology, Rikshospitalet Univ. Hospital and Univ. of Oslo, Oslo, Norway; 2) Dep. of Pediatrics, Ullevål Univ. Hospital, Oslo, Norway; 3) Div. of Epidemiology, Norwegian Inst. of Public Health, Oslo, Norway; 4) Inst. of Medical Genetics, Faculty Div. Ullevål Univ. Hospital, Univ. of Oslo, Norway; 5) Inst. of Immunology, Rikshospitalet Univ. Hospital, Oslo, Norway.

Nuclear Factor-B1 (NFB1) encodes two proteins, one of which (p50) is a transcription factor involved in regulation of both adaptive and natural immune responses, as well as a number of non-immunological responses. Significant changes in expression and function of NFB1 have been shown in studies of non-obese diabetic (NOD) mice, a murine model of type I diabetes (T1D) in humans. A -94delATTG promoter allele has been shown to cause lower promoter activity in reporter gene assays. The opposite allele (-94insATTG) has been reported associated with T1D in North American and UK families. In an effort to replicate these findings, 434 Norwegian T1D trio families were genotyped using fragment length analysis. Preliminary results, before confirming the genotyping results with an alternative method, shows a moderately significant positive association with the -94insATTG allele (RR = 1.276/p = 0.01933 by the transmission disequilibrium test (TDT) and OR = 1.264/p = 0.02098 by the test of haplotype-based haplotype relative risk (HHRR)). Together with earlier findings, these results strengthen the possibility that NFB1 is involved in the development of type I diabetes in humans.

In addition to the results on NFB1, genotyping results in the same material of a functional FCRL3 polymorphism (-169 C/T) will be presented. FCRL3 is a member of the Fc receptor-like family, with as yet unknown functions. However, FCLR3 lies in a region (1q21-23) showing linkage to multiple autoimmune diseases. The -169 C/T polymorphism alters the binding affinity for NFB1 and regulates FCLR3 expression, and has recently been reported associated with several autoimmune diseases in a Japanese population.

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Association of the PPAR P12A polymorphism with type 2 diabetes in the Saudi population. *S.M. Wakil[1], O. Alsmadi[1], K. Al-Rubeaan[2], F. Imtiaz[1], P. Carroll[1], M. Rajab[1], S. Al-Katari[2], M. Al-Katari[2], B.F. Meyer[1].* 1) Aragene, KFSH&RC, Riyadh, Central, Saudi Arabia; 2) Diabetes Center, King Abdulaziz University Hospital, Riyadh,Saudi Arabia.

The Peroxisome Proliferator-activated receptor- (PPAR) is a ligand-activated transcriptional factor involved in lipid and glucose metabolism, fatty acid transport, and adipocyte differentiation. PPAR has a common polymorphism designated Pro12Ala (P12A) that leads to a non conservative change from proline to alanine at codon 12. In a study conducted on Japanese and Finns, the P12A allele was found to associate with metabolically deleterious phenotypes, such as decreased insulin sensitivity, obesity, and type 2 diabetes. However, in other studies in Caucasians, no similar association was found. To date there are no reports regarding the association of P12A polymorphism with type 2 diabetes in Arab populations. We developed a molecular beacon based real time PCR assay for the genotyping of this polymorphism. We studied a group of 1348 patients diagnosed with type 2 diabetes based on WHO criteria and 219 normal control subjects (age >60 with fasting blood glucose <7mM). The PPAR P allele frequencies were 0.96 and 0.94 in type 2 diabetics and controls respectively. The PPAR A allele frequencies were 0.04 and 0.06 in type 2 diabetics and controls respectively. P12A allele frequencies were not significantly different in type 2 diabetics and controls (p=0.164). The very high incidence of the risk allele in this population (0.96) requires further analysis of a much larger sample population to identify relative risk significance. The risk allele is present in the Saudi population at one of the highest frequencies observed in any study. Whilst risk associated with P12A may contribute to development of type 2 diabetes in this population, the relative risk (diabetics vs normals) is low and indicates the likely presence of other genes and/or environmental factors which may have a greater influence upon the development of type 2 diabetes in this population.

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Modest T2D association of HNF4A P2 promoter SNPs observed in meta-analysis of more than 18,000 T2D individuals and controls. *L. Scott[1], K. Mohlke[2], F.S. Collins[3], M. Boehnke[1], for the HNF4A Meta-Analysis Consortium.* 1) U Michigan, Ann Arbor, MI; 2) UNC, Chapel Hill, NC; 3) NHGRI, NIH, Bethsda, MD.

SNPs near the P2 promoter of HNF4A were previously reported to be associated with type 2 diabetes (T2D) in Finnish (FUSION) and Ashkenazi Jewish samples (Silander et al. 2004 and Love-Gregory et al. 2004, multiplicative OR (95%C.I.) of 1.33 (1.06-1.65) and 1.46 (1.12-1.91), respectively). Now we have performed a meta-analysis of published and unpublished data for HNF4A SNPs genotyped in 19 additional case/control and 4 family-based samples. These samples consist of 9852 T2D individuals and 8833 controls from European, European-American, Hispanic, African-American, African, and American Indian backgrounds. We combined odds ratios (ORs) weighted by sample size for 4 associated P2 region SNPs observed to be in almost perfect LD in the original studies (rs4810424, rs1884613, rs1884614, rs2144908). The meta-analysis multiplicative OR for the non-original samples was 1.064 (95% C.I. 1.015-1.116, p=.01), in the direction of the original associations although considerably smaller. Including the FUSION or Ashkenazi samples increased the OR to 1.074 (95% C.I. 1.026-1.125, p=.003) and 1.074 (95% C.I. 1.026-1.135, p=.003), respectively. However, we observed significant heterogeneity in ORs for each analysis (p=.002, .001, and .0007, respectively). We next tested for study-wide correlation between the T2D OR and family history, study type, ethnicity, mean sample BMI, and mean sample age of diagnosis. Only older mean age of diagnosis showed a nominal trend toward association with the original risk alleles (p=.07). In addition, 8 HNF4A SNPs outside the P2 region were genotyped in at least 3 studies including the original ones. Of these, the C allele of rs3212183 (OR=1.15, 95% C.I. 1.04-1.28, p=.007, n studies =5) and the C allele of rs6031552 (OR=1.16, 95% C.I. 1.01-1.33, p=.04, n studies =3) showed significant association with T2D. Overall we found modest evidence for association of HNF4A SNPs with T2D in a large meta-analysis, and further observed significant between sample heterogeneity suggesting there may be sample-specific characteristics influencing the effect of HNF4A variants.

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Contribution of Common PBX1 SNPs to Type 2 Diabetes Susceptibility in French Caucasians. *K. Duesing[1], P. Froguel[1,2], F. Gibson[1], International Type 2 Diabetes 1q Consortium.* 1) Imperial College London, UK; 2) CNRS 8090, Institut Pasteur de Lille, France.

Pre-B-cell leukemia transcription factor 1 (Pbx1) is a member of the TALE class of homeodomain proteins, which function as components of hetero-oligomeric transcription factor complexes. The interaction of Pbx1 with Pdx1, a master regulator of pancreatic development, has been shown to be essential for the normal proliferation of differentiated pancreatic cells and Pbx1 deficient mice exhibit pancreatic hypoplasia. The human PBX1 gene is located at chromosome 1q23, within the most consistently replicated type 2 diabetes (T2D) susceptibility locus in the human genome. We have examined the contribution of common PBX1 SNPs (frequency5%) to T2D susceptibility in the French Caucasian population. Sequencing of 1kb of the PBX1 promoter region, exons and flanking intronic sequence in 24 individuals identified 12 SNPs. In addition, we selected 21 SNPs from dbSNP, of which 18 were in ultra-conserved non-coding regions. These 33 SNPs were genotyped in 744 T2D cases and 894 normoglycemic controls. We identified 4 SNPs in intron 2 (P=0.046-0.006) and a non-synonymous coding SNP (G21S) in exon 1 (P=0.028) that were associated with T2D. Interestingly, the G21S variant is located in the Meis1 interaction domain of PBX1 that has been shown to influence its nuclear localisation. We employed various methods (linkage partitioning, GIST, LAMA) to analyse the associated SNPs for contribution to the linkage signal at 1q in French T2D families. The results of these analyses consistently indicated that the G21S variant, but not the intron 2 SNPs, is associated with the evidence for linkage (P=0.02-0.03). Furthermore, we observed that the frequency of the associated S allele increases progressively from controls (0.20)cases (0.23)probands from T2D families (0.35)probands from families showing linkage to 1q (0.42). In summary, common variation in the PBX1 gene is both associated with T2D and contributes to the linkage signal at 1q in the French Caucasian population.

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Insulin Promoter Factor 1 Variation is Associated with Type 2 Diabetes in African Americans. *S.C. Elbein[1,2], M.A. Karim[1], T.C. Hale[1,2], X. Wang[1,2].* 1) Division of Endocrinology and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Medical Service, Central Arkansas Veterans Healthcare System, Little Rock, Arkansas.

Defective insulin secretion is a key defect in the pathogenesis of type 2 diabetes (T2DM). The -cell specific transcription factor, insulin promoter factor 1 gene (IPF1), is essential to pancreatic development and the maintenance of -cell mass. We hypothesized that regulatory or coding variants in IPF1 contribute to T2DM. We screened 71 Caucasian and 69 African American individuals for genetic variants in the promoter region, three highly conserved upstream regulatory sequences (PH1, PH2 and PH3), the human -cell specific enhancer, and the 2 exons with adjacent introns. We identified 8 variants, including a 3 bp insertion in exon 2 (InsCCG243) in African Americans that resulted in an in-frame proline insertion in the transactivation domain. No variant was associated with T2DM in Caucasians (192 cases and 192 controls). However, SNPs at -3766 in the human -cell enhancer, at -2877 bp in the PH1 domain, and at -108 bp in the promoter region were associated with T2DM in African American subjects (341 cases and 186 controls; $p<0.01$), both individually and as haplotypes ($p=0.01$ correcting by permutation test). No SNP altered a binding site for the expected -cell transcription factors. The rare alleles of InsCCG243 in exon 2 showed a trend to over-representation among African American diabetic subjects ($p<0.1$). The common alleles of regulatory variants in the 5' enhancer and promoter regions of the IPF1 gene increase susceptibility to type 2 diabetes among African American individuals, likely as a result of gene-gene or gene-environment interactions. In contrast, IPF1 is not a cause of type 2 diabetes in Caucasians. The InsCCG243 variant was previously shown to alter insulin secretion in cell lines, is present in up to 20 percent of African American individuals with T2DM, and may contribute to diabetes susceptibility in this population. However, this coding variant is of much lower penetrance than previously suggested.

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FOXO1A as a Candidate Gene for Type 2 Diabetes. *M. Karim[1], X. Wang[2,3], R.L. Craig[2,3], S.C. Elbein[2,3].* 1) Pediatrics, Arkansas Children's Hospital, Little Rock, AR; 2) Division of Endocrinology and Metabolism, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 3) Medical Service, Central Arkansas Veterans Healthcare System, Little Rock, AR.

The human forkhead box O1A (FOXO1A) gene, located on chromosome 13q14.1, is a key transcription factor in insulin signaling in liver and adipose tissue and controls key pancreatic beta cell genes including IPF1. Thus, sequence variants of FOXO1a could contribute to defects in hepatic and peripheral insulin action and altered beta cell compensation that characterize type 2 diabetes (T2DM). To test this hypothesis, we used a combination of DHPLC and fluorescent sequence analysis to screen for sequence variants in the three exons and 3' untranslated region, and 1.5 kb of the 5' flanking region for sequence variants in Caucasian and African American individuals with early onset (<45 years) T2DM. We identified 6 variants, but none altered the coding sequence and all but one in the 3 untranslated region was rare or absent in Caucasians. We also selected 7 SNPs in the large first intron and 5 flanking region. Together, the 13 SNPs spanned 116.4 kb. We selected 8 SNPs to type in a Caucasian population comprising 192 unrelated nondiabetic control individuals and 192 individuals with T2DM, and 10 SNPs to type in 182 controls and 352 diabetic individuals of African American ancestry. No variant was associated with T2DM (African Americans, p>0.08; Caucasians, p> 0.09). Of the 8 Caucasian SNPs, 6 fell in a single haplotype block spanning over 100 kb and including most of the large first intron. In contrast, only SNPs at -7747 and -54 formed a block in African Americans. No haplotype was associated with T2DM. FOXO1 variation is rare, and is unlikely to contribute to T2DM in either Caucasian or African American populations.

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Association study of two polymorphisms from endothelial nitric oxide synthase gene in a cohort of West African diabetic subjects and controls: The ADDM study. *Y. Chen[1], H. Huang[1], A. Doumatey[1], T. Aje[1], J. Zhou[1], G. Chen[1], A. Adeyemo[1,2], A. Amoah[6], J. Acheampong[3], J. Oli[5], B. Osotimehin[2], T. Johnson[4], F. Colins[7], G. Dunston[1], C. Rotimi[1].*
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Endothelial nitric oxide synthase (eNOS) plays a key role in regulating vascular tone. The Glu298Asp (G/T) substitution of eNOS gene has been reported associated with coronary heart disease and hypertension. Another polymorphism, a 27bp insertion/deletion polymorphism was also reported associated with hypertension, type 2 diabetes, diabetic retinopathy and nephropathy. We genotyped 385 subjects with type 2 diabetes and 191 controls from five West Africa centers for both the G/T polymorphism and the insertion/deletion polymorphisms. No association was found between the two polymorphisms and type 2 diabetes or hypertension. However, we found that the GG genotype was significantly associated with increased creatinine clearance in diabetic subjects($p=0.043$) and diabetic subjects with hypertension($p=0.034$). The aa genotype was significantly associated with increased creatinine clearance in diabetic subjects with retinopathy. The bb genotype of the insertion/deletion polymorphism is also significantly associated with diabetes retinopathy with OR=2.4(95% CI=1.39-4.09). In conclusion, both of the polymorphisms studied were associated with increased creatinine clearance but not associated with type 2 diabetes or hypertension; Whereas, the insertion/deletion polymorphism was associated with both diabetic retinopathy and increased creatinine clearance in this West African cohort.

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Association of the Thr92Ala variation in the DIO2 gene with young-onset diabetes in Pima Indians. *S. Nair, YL. Muller, E. Ortega, S. Kobes, C. Bogardus, L. Baier.* Phoenix Epidemiology and Clinical Research Branch, National Institute of Health, Phoenix, AZ.

The Pima Indians of Arizona have a high prevalence of obesity and type 2 diabetes mellitus (T2DM), and physiologic candidate genes that might explain increased susceptibility for these diseases are being investigated. The Type 2 Deiodinase (DIO2) gene encodes a deiodinase (DIO2), that converts the thyroid pro-hormone thyroxine (T4) to the more active form triiodothyronine (T3). Since thyroid hormones have important roles in the regulation of energy balance (in particular by stimulating thermogenesis) and may also influence glucose metabolism, variation in the DIO2 gene could affect susceptibility to obesity and /or T2DM. Indeed a Thr92Ala variation in DIO2 has previously been reported to be associated with insulin resistance in non-diabetic Caucasian women and in patients with T2DM. We have genotyped this variation in a diabetes case vs. control group of non-first degree related Pima Indians (146 diabetic subjects with an age of onset 25years vs. 147 non-diabetic subjects who are at least 45 years of age). The Thr92Ala (minor allele frequency = 0.19) was associated with T2DM ($p = 0.01$, odds ratio = 1.7, 95% CI = 1.1-2.6, additive model). However, in an obesity case vs. control group (362 obese subjects with a mean BMI = 51.7 6.2 vs. 127 lean subjects with mean BMI = 26.3 2.7) there was no association of the Thr92Ala with obesity, nor was there an association of this variant with BMI in a family-based association analysis (N = 1200). The Thr92Ala was further analyzed for associations with metabolic predictors of T2DM in 256 non-diabetic, full-heritage Pima subjects who had undergone detailed metabolic testing. The Thr92Ala was modestly associated with total triglyceride concentrations and hepatic glucose output ($p = 0.04$ and $p = 0.02$, respectively, adjusted for age, sex, percent fat and nuclear family membership). We are currently scanning the DIO2 gene by direct sequencing to search for additional polymorphisms that might substantiate an association between the DIO2 gene and T2DM.

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Genotyping of 4608 SNPs across a 10Mb region of chromosome 20q in four UK and an Ashkenazi population suggests additional type 2 diabetes (T2D) candidate genes. *I. Barroso[1], J. Luan[2], P. Whittaker[1], E. Zeggini[3], S. Hunt[1], N. Walker[4], B. Glaser[5], A. Hattersley[6], M.A. Permutt[7], M. McCarthy[3], P. Deloukas[1], N.J. Wareham[2].* 1) The Wellcome Trust Sanger Institute, Hinxton, UK; 2) MRC Epidemiology Unit, Cambridge, UK; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 4) JDRF/WT Diabetes and Inflammation Laboratory, Cambridge, UK; 5) The Hadassah Hebrew University Medical Center, Israel; 6) Peninsula Medical School, Exeter, UK; 7) Washington University School of Medicine, St. Louis, MO, USA.

Susceptibility to T2D has been identified in a broad region of chromosome 20q by genome scans in Caucasian, Ashkenazim, Chinese Han, Japanese and West Africans. SNPs in the proximal promoter region of *HNF4A* have recently been shown to associate with T2D predisposition in Caucasian and Ashkenazi populations. It remains unknown whether *HNF4A* explains all of the linkage signal or whether additional genes on 20q contribute to T2D susceptibility. We used a dense linkage disequilibrium (LD) map of chromosome 20 constructed at the Sanger Institute to select SNPs from 10Mb within the linkage peaks, for genotyping in four UK and an Ashkenazi population. We typed 4608 SNPs (38.1Mb-48.2Mb NCBI build 35, 1 SNP per 2Kb on average) in 1231 T2D patients and 1277 controls using the Golden Gate assay (Illumina platform). Meta-analysis for single SNP associations was performed in the four UK populations and 704 SNPs were shown to associate with T2D risk with $p<0.15$. A number of SNPs in *HNF4A* were associated at this significance level in the UK and Ashkenazi populations. More interestingly, a number of SNPs clustered in different genes (between 40.1Mb-41.7Mb) were more significantly associated with T2D in all populations than *HNF4A* SNPs. These 704 SNPs as well as 50 SNPs that associated with T2D risk in Ashkenazim ($p<0.01$) and that were not associated in the four UK populations at $p<0.15$ have been selected for Phase II of this study. Phase II will comprise replication testing of these SNPs in an independent group of samples comprising approximately 1300 cases and 1300 controls from the same populations used in Phase I.

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Association of the Gly482Ser Polymorphism in the Peroxisome Proliferator-Activated Receptor Coactivator-1 with Excess Weight Gain by Male Subjects with Type 1 Diabetes upon Intensive Diabetes Therapy. *S.S. Deeb*[1], *J.D. Brunzell*[2], *DCCT/EDIC Investigators.* 1) Dept Medicine/Med Genetics, Univ Washington, Seattle, WA; 2) Dept Medicine/Endocrinology, Metabolism, and Nutrition, Univ Washington, Seattle, WA.

The Diabetes Control and Complications Trial (DCCT) involved intensive diabetes therapy for an average period of 6.5 years. A subset of these patients gained excessive weight, mainly due to accumulation of visceral fat. Patients with a family history of type 2 diabetes mellitus (T2DM) were among those who gained more weight. To identify genetic factors that contribute to excessive weight gain in these patients, we investigated the association of one sequence variant in each of 7 candidate genes for association with this trait by comparing allele and genotype frequencies between quartile 1 (low weight gain) and quartile 4 (high weight gain). The Gly482Ser polymorphism in the gene encoding the peroxisome proliferator-activated receptor coactivator-1 (PGC-1) was the only one that was significantly associated with weight gain in men ($p = 0.0045$) but not in women. The Ser allele was associated with more weight gain than the Gly allele ($p = 0.005$). Those with the Gly/Gly genotype were overrepresented in quartile 1 ($p = 0.0054$), whereas carriers of the Ser allele (Gly/Ser and Ser/Ser) were overrepresented in quartile 4 ($p = 0.013$). These data support the previous findings that genetic variation in PGC1 may modify the effects of physiological and environmental factors that predispose to the metabolic syndrome and T2DM.

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A novel -192c/g mutation in the proximal P2-promoter of *HNF4A* co-segregates with early onset diabetes. *J. Ek[1], S.K. Hansen[1], M.L. Jensen[1], S. Pruhova[2], A. Johansen[1], T.W. Boesgaard[1], A. Albrechtsen[1], J. Lauenborg[3], M. Parrizas[4], S.F. Boj[4], L. Bjørkhaug[5], P.R. Njølstad[5], T. Jørgensen[6], K. Borch-Johnsen[1,6,7], P. Damm[3], J. Ferrer[4], J. Lebl[2], O. Pedersen[1,7], T. Hansen[1].* 1) Steno Diabetes Center, Gentofte, Denmark; 2) Department of Pediatrics, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 3) Department of Obstetrics, Rigshospitalet, Copenhagen, Denmark; 4) Endocrinology unit, Hospital Clinic Universitari, Institut dInvestigacions Biomédiques August Pi I Sunyer, Barcelona, Spain; 5) Section for Medical Genetics and Molecular Medicine, Department of Clinical Medicine, University of Bergen, Norway; 6) Research Centre for Prevention and Health, Glostrup University Hospital, Denmark; 7) Seventh Faculty of Health Science, University of Aarhus, Denmark.

Recently, it has been shown that mutations in the P2-promoter of the -cell expressed isoform of hepatocyte nuclear factor-4 (*HNF4A*) cause MODY. In this study we have examined the P2-promoter and the associated exon 1D of *HNF4A* for variations associated with various forms of early-onset non-autoimmune diabetes. We examined 1189 bp upstream of the ATG site of the P2-promoter and exon 1D in 114 T2D patients of whom 47 patients had age at diagnosis < 40 years, 72 MODYX probands and 85 women with previous gestational diabetes (GDM). A -192c/g mutation was found in one MODY proband and in one early-onset T2D patient, and in 2 GDM probands. Also, the variant co-segregated with diabetes in the GDM families. Carriers of the -192c/g mutation had an impaired glucose stimulated insulin secretion compared to matched diabetic subjects and the insulinogenic index was significantly decreased (p=0.02). Two novel completely linked polymorphisms in the P2-promoter at positions -1107g/t and -858c/t, respectively, were identified. There was no difference in allelic frequency of the variants among 1430 T2D patients and 4812 normal glucose tolerant (NGT) subjects. In conclusion, a novel -192c/g mutation of *HNF4A* are a rare cause of early-onset diabetes among Danish Caucasians, highlighting the importance of this region when investigating the genetics behind diabetes.

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Fine Mapping of a Putative Type II Diabetes Susceptibility Gene on 1q in Native American Pimas. *J.M. Singer[1], V. Ossowski[1], M.I. McCarthy[2], P. Deloukas[2], E. Zeggini[2], W. Rayner[2], C. Bogardus[1,2], M. Prochazka[1,2].* 1) Phoenix Epidemiology and Clinical Research Branch NIDDK NIH, Phoenix, AZ; 2) International Type II Diabetes 1q Consortium.

Previous genome-wide scans in different populations (including Native American Pimas, Caucasians, and Chinese) detected linkage of chromosome 1q21-q25 with type 2 diabetes mellitus (T2DM). Currently, an international collaborative effort (International Type II Diabetes 1q Consortium) is underway to fine map this region as a first step in detecting causative genes and mutations contributing to T2DM. So far, analysis of over 2000 SNPs in Pima samples has revealed 3-4 loci potentially associated with the disease. One association signal is at 159.9 Mb (NCBI build 35), between known genes *RGS5* and *CDCA1*. Family based association p-values for these SNPs range from 0.05-0.0009 (T2DM onset before the age of 45yr), and the original LOD score (2.6) is reduced by up to 20% when the linkage analysis is conditioned on SNP genotypes. The region flanked by *RGS5* and *CDCA1* contains at least three additional UniGene clusters, representing transcripts with unknown function. We have performed deep resequencing of the exons and promoter regions of these transcripts, and also included *RGS5* and *CDCA1* in our analysis. The most significantly associated SNPs are clustered within 60 kb, and do not overlap with either *RGS5* or *CDCA1*. Several of them are located in exons of the novel UniGene transcripts and have the potential of altering the predicted ORFs. We have also determined the expression profiles of these UniGene transcripts by PCR using cDNAs from 16 various organs and tissues, and they appear to be ubiquitously distributed. A complete deep resequencing of approximately 70 kb encompassing the significantly associated area is in progress to define all variants in this region and complete a thorough association analysis.

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Fine mapping and association study for Graves' disease in Chinese Hans. *X. Chu[1], L. Sun[1], R. Lei[1], Z. Niu[1], L. Xu[1,3], K. Zhang[1,3], Y. Wang[1], Y. Dong[1], Y. Wang[1], H. Song[2], Z. Chen[1,2], L. Jin[1,4], W. Huang[1].* 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) Ruijin Hospital, SSMU, Shanghai, China; 3) Shanghai South Gene Technology Co., Ltd., Shanghai, China; 4) Institute of Genetics, Fudan University, Shanghai, China.

Graves' disease (GD) is an organ-specific autoimmune disorder and its etiology is believed as the results of interaction of inheritance and environment. Despite the human leukocyte antigen and cytotoxic T lymphocyte antigen 4 have been reported with a strong association with the GD trait, more susceptibility genes observed to be associated with its pathogenesis remain to be revealed. To identify susceptibility genes on chromosome 5q31, we carried out a genome-wide scan on 322 individuals from 54 Chinese Han multiplex GD pedigrees combined with a SNP genotyping design, leading to the fine mapping and the identification of a risk-conferring SNP haplotype. The result showed the strongest evidence for linkage at D5S436 on chromosome 5q31. A fine mapping using four additional markers around D5S436 was then performed and a maximum multipoint LOD score of 4.12 were obtained for marker D5S2090, among which regions containing several interleukin genes cluster arose our interests. Finally, 20 candidate genes including all the cytokine genes in 40Mb region flanking D5S2090- D5S436 were resequenced in a set of 48 unrelated Chinese Han subjects and more than 200 SNPs have been identified. Using LD analysis, we selected 50 tagSNPs and genotyped them in 380 cases and 690 controls collected from China. A nonsynonymous SNP(P27S) and another SNP at nt ¨C16 (T/C) in the 5' flanking region of the same gene showed a strong association with the disease. The further related investigation to explain function and interaction of SNPs in pathogenesis of Graves' disease will be continued.

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Variation in PDGF-C and PDGFR and Nonsyndromic Cleft Lip and Palate. *B.T. Chiquet[1], S.H. Blanton[2], J.T. Hecht[3].* 1) University of Texas Dental Branch at Houston; 2) University of Virginia, Charlottesville; 3) University of Texas Medical School at Houston.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common birth defect with a birth prevalence of 1/700 live births. NSCLP is a complex disorder postulated to be caused by multiple genes and environmental factors. The region on chromosome 4q31 to 4qter has been implicated in multiple genome scans to harbor a clefting gene. Platelet-derived growth factor C (PDGF-C) on chromosome 4q32 and its receptor, platelet-derived growth factor receptor alpha (PDGFR) on chromosome 4q12, both play a role in normal palate fusion and epithelial degradation and are biological plausible candidate genes. Variation in these genes may play a role in NSCLP. Single nucleotide polymorphism (SNP) analysis of PDGF-C and PDGFR was undertaken using TaqMan SNP Genotyping Assays. Six SNPs in PDGF-C and five SNPs in PDGFR were selected for this study using 51 multiplex families and 184 simplex parent-child trios. All SNPs in both genes were in Hardy-Weinberg equilibrium. The SNPs within PDGFR were largely in linkage equilibrium, while most of the SNPs in PDGF-C were in extreme linkage disequilibrium. Nonparametric analysis of the SNPs with Genehunter yielded an NPL score of 1.4 ($p=0.05$) for one of the SNPs in PDGF-C. Transmission disequilibrium analysis with PDT found suggestive evidence for an altered transmission of two SNPs in PDGFR under a genotypic model ($p=0.0485$ and $p=0.0406$) and for one of the same SNPs under a generalized model ($p=0.01$). These results suggest that PDGF-C and PDGFR may play a role in nonsyndromic clefting and warrants additional studies.

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A gene for speed: The *ACTN3* R577X polymorphism influences human muscle performance. *D.G. MacArthur[1], N. Yang[1], A.J. Kee[2], E.K. Kettle[2], J. Hook[3], F. Lemckert[3], E.C. Hardeman[2], P.W. Gunning[3], K.N. North[1].* 1) Neurogenetics Research Unit, Children's Hospital at Westmead, Sydney, NSW, Australia; 2) Muscle Development Unit, Children's Medical Research Institute, Sydney, NSW, Australia; 3) Oncology Research Unit, Children's Hospital at Westmead, Sydney, NSW, Australia.

The protein -actinin-3, encoded by the *ACTN3* gene, is a highly conserved component of the contractile machinery in fast skeletal muscle fibres. Intriguingly, a common nonsense variant in the human *ACTN3* gene (R577X) results in complete deficiency of -actinin-3 in ~18% of the general population who are homozygous for the X allele. We have recently demonstrated a significantly lower frequency of XX homozygotes amongst elite Australian sprint athletes than controls, suggesting that the presence of -actinin-3 is required for optimal fast fibre function. This finding has been supported by a number of recent studies demonstrating associations between R577X genotype and muscle strength and response to training. The *ACTN3* R577X polymorphism thus represents a genetic factor influencing muscle performance in humans.

We are currently investigating the evolutionary history of the 577X allele in humans. Analysis of the pattern of genetic variation in the R577X region in different ethnic groups suggests that the X allele is ancient and was maintained at low frequency, possibly by balancing selection, in the African ancestors of modern humans. The high frequency of 577X in many non-African ethnic groups may indicate more recent positive selection on the X allele in these populations.

We have also generated an *Actn3* knockout mouse to explore the mechanisms by which -actinin-3 deficiency affects muscle function. The loss of -actinin-3 results in the upregulation of a closely related protein, -actinin-2, in fast muscle fibres, which may partially compensate for the deficiency of -actinin-3. However, a marked increase in sarcomeric damage following eccentric exercise in knockout mice compared to wild-type suggests that -actinin-3 protects muscle from exercise-induced damage, potentially explaining the association between R577X and muscle function in humans.

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Gender stratified linkage analysis for premature coronary artery disease (CAD). *B.R. Pedersen[1], C. Haynes[1], D. Crossman[2], J. Haines[3], C. Jones[4], W.E. Kraus[1], E.R. Hauser[1].* 1) Duke University Medical Center, Durham, NC; 2) University of Sheffield, Sheffield, UK; 3) Vanderbilt University, Nashville, TN; 4) University of Wales, Cardiff, UK.

Gender and family history are well-known risk factors for CAD, particularly when combined with an early age of onset. There is also a suggestion that some genetic effects may be gender-specific (McCarthy et al. Hum Genet. 114:87-98). We examined whether differences in linkage exist between gender-stratified affected sibling pairs (ASPs). A genomewide scan of linkage using 395 microsatellite markers was performed in 493 ASPs from 420 families. Families were stratified into 61 female, 279 male, and 157 mixed gender ASPs. Affected status was defined as CAD before the age of 51 years for males and 56 years for females. Evidence for linkage to specific regions was taken as nonparametric multipoint LOD score>1.0. LOD scores were reported at DECODE cM and were specific to the results for overall, male, female, and mixed gender pairs (LOD, MLOD, FLOD, and XLOD, respectively). We continue to observe evidence for linkage on chromosome 3 (Hauser ER et al. Am. J. Hum. Genet. 75:436-47) but not specific to gender (LOD=2.58, 134.5 cM; MLOD=0.97, 150.4 cM; FLOD=0.78, 132.2 cM; XLOD=0.72, 213.8 cM). Enhanced peaks were found on chromosome 5 in males (LOD=1.31, 200.8 cM; MLOD=1.91, 208.0 cM; FLOD=1.18, 138.0 cM; XLOD=0.39, 173.7 cM). Only females and overall pairs demonstrated linkage on chromosome 7 (LOD=1.30, 83.0 cM; MLOD=0.61, 39.1 cM; FLOD=1.99, 39.1 cM; XLOD=0.91, 80.7 cM). New linkage peaks were observed on chromosomes 6 for both genders, roughly 35 cM apart (LOD=0.92, 158.7 cM; MLOD=1.39, 157.5 cM; FLOD=1.93, 192.0 cM; XLOD=0.47, 73.9 cM). Chromosomes 8 (MLOD=1.19, 97.3 cM), 12 (LOD=1.0, 121.8 cM), 14 (FLOD=1.24, 4.5 cM), and 18 (XLOD=1.18, 49.6 cM) also revealed new evidence for linkage. These results indicate variable genetic effects by gender for premature CAD, reflecting the sampling scheme or genetically predicted CAD risk factors that differ between men and women such as HDL, LDL, and hypertension.

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A PDFGC regulatory region SNP that decreases PDGFC promoter transcriptional activity is associated with CL/P. *T.C. Hart[1], S.J. Choi[1], P.S. Hart[2], P.P. Sulima[1], M.L. Marazita[3].* 1) Section on Craniofacial & Dental Genetics, NIDCR, NIH, Bethesda, MD; 2) Office of the Clinical Director, NHGRI, NIH, Bethesda, MD; 3) University of Pittsburgh, Pittsburgh, PA.

The murine pdgfc-/- knockout is associated with a complete cleft of the secondary palate, demonstrating PDGF-C is necessary for palate formation. Human linkage and association studies support a gene(s) for nonsyndromic cleft lip with or without cleft palate (CL/P) on chromosome 4q31-q32 at or near the PDGFC gene locus. Linkage support is greatest in Chinese families. To evaluate the role of PDGFC gene mutations in CL/P Methods: We sequenced the PDGFC gene in 108 Chinese CL/P cases and 113 controls to evaluate its role in human CL/P. We also performed transfection assays of PDGFC four different promoter constructs to evaluate the effect of a novel -986C>T regulatory region SNP on PDGFC promoter activity. Results: No coding region mutations were identified, however a novel -986C>T SNP was significantly associated with CL/P (p=0.01) in Chinese cases from families that showed evidence of linkage to 4q31-q32. Database search demonstrate that the -986T allele abolished six overlapping transcription factor regulatory motifs (Sp1, USF, WT1, EGR-1, HEB, and ETF). Transfection assays of PDGFC promoter reporter constructs in 3 different cell types indicate that the -986T allele is associated with a significant decrease (up to 80 percent) of PDGFC transcriptional activity. Conclusion: A novel -986C>T SNP in the PDGFC promoter is significantly associated with human CL/P in Chinese cases from families linked to 4q32.1. This promoter polymorphism disrupts a potential cytosine-rich regulatory motif and is associated with a significant decrease in transcription of PDGFC reporter constructs. This functional polymorphism acting on a susceptible genetic background may provide a component of CL/P etiology in humans.

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Dementia in the Amish: A follow-up linkage study. *J.L. McCauley[1], A.E. Crunk[1], L.L McFarland[1], P.C. Gaskell[2], L. Jiang[1], P.J. Gallins[2], W.K. Scott[2], K.A. Welsh-Bohmer[3], S.R. Johnson[3], C.E. Jackson[4], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine; 3) Joseph & Kathleen Bryan ADRC/ Division of Neurology, Duke University Medical Center, Durham, NC; 4) Scott & White, Temple, TX.

Alzheimers disease (AD) is the leading cause of dementia in the elderly. The underlying genetics of AD has proven to be challenging to understand and elucidate. While a role for the APOE gene in late-onset AD is clear, it accounts for less than half of the susceptibility and thus other genetic factors are likely to be involved. Genetic heterogeneity is a major complicating factor hindering further gene identification. With this in mind, we previously performed a genomic linkage screen using microsatellite markers on families ascertained from the genetically isolated, well-defined Amish population. Due to the complexity of these pedigrees, we employed both linkage analysis and combinatorial mismatch scanning. These approaches identified several regions of interest across the genome. Within these regions we have performed follow-up linkage in a set of 364 individuals (82 with AD) using single nucleotide polymorphisms. Regions demonstrating 2-pt lod scores 1.0 in the follow-up analysis included chromosome 3q(~209cM) at markers rs710446(Lod=1.1), rs1136644(Lod=1.5), and rs2169244(Lod=1.2) and chromosome 5q(~183cM) at marker rs628857(Lod=1.9). Our most promising results lie on chromosome 4q, which contains nine markers (rs2174080,rs3755896, rs2952886, rs1396009, rs1014107, hcv1718090, rs6826476, rs1993656, rs1388083) between 130-150 cM demonstrating average lod scores of 1.7. Interestingly, the most studied regions on chromosomes 9, 10, and 12 show little to no evidence of linkage in these families. However, our 3q, 4q, and 5q results do overlap with previous findings from other genome scans in AD. Our findings suggest that these regions harbor yet unconsidered susceptibility genes, and that the many previously reported regions of linkage should not be ignored.

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Candidate Gene Studies of Dementia in the Amish. *N. Schnetz-Boutaud[1], J.L. McCauley[1], A.E. Crunk[1], L.L. McFarland[1], P.C. Gaskell[2], L. Jiang[1], P.J. Gallins[2], W.K. Scott[2], K.A. Welsh-Bohmer[3], S.R. Johnson[3], C.E. Jackson[4], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine; 3) Joseph & Kathleen Bryan ADRC/ Division of Neurology, Duke University Medical Center, Durham, NC; 4) Scott & White, Temple, TX.

Alzheimers disease (AD) is the leading cause of dementia in the elderly. While there is a strong genetic component and four genes have been identified, three of these (APP, PS1, PS2) are involved primarily in rare early-onset AD and the fourth (APOE) explains less than half of the more common late-onset Alzheimers disease (LOAD). One difficulty has been the underlying genetic heterogeneity. To reduce both genetic and environmental heterogeneity, we have been studying dementia in the Amish communities of northern Indiana and central Ohio. The Amish are socially and genetically isolated from the surrounding population and have more homogeneous education and environmental exposures.

There have been over 90 candidate genes in AD with at least one positive report of association. We investigated nine of these candidate genes (AGT, NCSTN, LTA, UBQLN, GAPD, PZP, LIPC, ACE, APOE) in a sample of 364 Amish individuals (82 with AD) in five large pedigrees. A total of 40 SNPs were genotyped. Genetic linkage (using SIMWALK v. 2.0) gave modestly interesting lod scores for AGT (Lod=1.0), UBQLN (Lod=1.4), LIPC (Lod=1.9) and APOE (Lod=2.26)(see Virdagamo et al., this conference). The AGT and APOR results were derived primarily from one very large pedigree centered in Ohio. Nominally significant association results were observed for AGT (p=0.03), NCSTN (p=0.05), GAPD (P=0.02), PZP (P=0.01), and LIPC (P=0.03). None of these survive a correction for multiple comparisons. Only AGT and LIPC gave interesting results in both the linkage and association tests, suggesting that these two genes should be investigated further.

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A Follow-Up Genome Scan in Lithium Responsive Bipolar Disorder Families. *I. Jaitovich Groisman[1], F. Mamdani[1], T. Hajek[2,5], P. Grof[3], G.A. Rouleau[4], M. Alda[1], G. Turecki[1].* 1) McGill University, Montreal, Quebec, Canada; 2) Dalhousie University, Halifax, Nova Scotia,Canada; 3) University of Ottawa, Ottawa, Ontario,Canada; 4) Montreal University, Montreal, Quebec,Canada; 5) Charles University, Prague, Czech Republic.

Attempts to identify susceptibility genes for bipolar disorder (BD) require confirmation. We have been using a pharmacogenetic strategy in the study of genetic factors involved in BD, which has been based on the investigation of subjects who respond to long-term treatment to lithium. There is substantial evidence suggesting that lithium response may help define a more homogeneous BD phenotype. We have previously published a genome scan in families selected through excellent lithium responders (Turecki et al. 2001). Here we report on a follow-up genome scan on a larger sample of families using a total of 811 microsatellite markers spaced at an average of 5 cM. The clinical sample consisted of 36 families with a total of 282 subjects (135 affected with BD or recurrent unipolar disorder). The data was analyzed by the lod score method and NPL analysis. Similar to our previous study, we have detected suggestive evidence of linkage (multipoint lod scores 1) in 6p, 21q, 4q and 9q regions, some of which have been also reported by other groups. In addition, we identified linkage on 2 chromosomal regions with multi point LOD scores 2: 3p24-22 and 3p12-11, and another one 3 at 14p13-11. We have not found additional support for linkage on 15q and 7q, as suggested by our first study. In conclusion, our data add to the view of BD as a heterogeneous condition associated with multiple susceptibility loci. Further fine mapping would allow for the identification and study of candidate genes involved in 14q13-11. References: Turecki G et al. (2001). Mapping susceptibility genes for bipolar disorder: a pharmacogenetic approach based on excellent response to lithium. Molecular Psychiatry 6: 570 - 578.

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A Novel Mutation in GCAP1 in Autosomal Dominant Cone Dystrophy. *Y. Zhao[1], X. Li[1], D. Birch[2], Z. Yang[1], J. Baird[1], D. Gibbs[1], D. Wheaten[2], K. Zhang[1].* 1) Department of Ophthalmology and Visual Science, and Program in Human Molecular Biology & Genetics, Eccles Institute of Human Genetics, University of Utah Health Science Center, Salt Lake City, UT 84112; 2) Retina Foundation of the Southwest, Dallas, TX 75231.

Purpose: The cone dystrophies are characterized by progressive dysfunction of the photopic (cone-mediated) system, presenting with photophobia, loss of color vision and reduced central visual acuity. Cone dystrophy is genetically heterogeneous and may present as an autosomal dominant, autosomal recessive, or X-linked recessive trait. In this study, we are trying to investigate the clinical features and gene mutations in one family with an autosomal dominant form of cone dystrophy. Methods: Ophthalmologic examinations were performed and genomic DNA extracted from bloods using the Puregene DNA isolation kit under consent. DNA samples were amplified by the polymerase chain reaction using primers corresponding to each exon of GCAP1. The PCR products were screened for mutations by direct sequencing. Results: Of 20 members in this family, with 5 generations, there are 5 affected patients. Clinical findings in this family include photophobia, decreased visual acuity and color vision abnormalities typical of cone dystrophy. Sequencing analysis revealed one novel mutation in exon2, which is at nucleotide C312A, and the corresponding amino acid change Asn104Lys. Conclusions: We identified a novel mutation in the GCAP1 gene in one family with autosomal dominant cone dystrophy. Genetic and functional studies of this mutation will aid our understanding of underlying pathogenetic mechanism.

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Distribution of DNA sequence variants in the WDR36 gene in primary open angle glaucoma patients. *R.R. Allingham[1], L. Linkroum[2], J. Wang[1], K. Larocque-Abramson[1], F.L. Graham[1], E.A. del Bono[2], J.L. Haines[3], M.A. Pericak-Vance[1], M.A. Hauser[1], J.L. Wiggs[2].* 1) Duke Univ Medical Ctr, Durham, NC; 2) Harvard Medical School, Boston, MA; 3) Vanderbilt University, Nashville, TN.

Primary open angle glaucoma (POAG) is a complex inherited disease that is likely to result from defects in multiple susceptibility genes as well as environmental factors. WDR36 is a WD40 repeat protein that may be involved in T cell activation. Recently DNA sequence variants in the WDR36 gene have been identified in patients with high and low tension glaucoma (Monemi et al., 2005, Hum Molec Genet 14:725). To evaluate the role of this gene in familial high tension primary open angle glaucoma, we screened the probands from a set of 86 adult-onset POAG families, as well as probands from 6 early-onset primary open glaucoma families. Criteria for inclusion include elevated intraocular pressure 22mm Hg in both eyes, optic nerve damage, and corresponding visual field loss in at least one eye. Adult onset probands had an age of onset after age 35, while early-onset probands had an age of onset before age 35. All of the 23 coding exons and flanking introns were sequenced in 92 probands and 88 age and ethnically matched controls. Pools of genomic DNA were initially sequenced using ABI sequencing chemistries and run on an ABI-3100 automated DNA sequencer. Pools displaying DNA sequence variants were resequenced. Nine nonsynonymous SNPs, 7 synonomous SNPs and 13 intronic variants were identified. None of the intron changes would be expected to affect splice sites. Four of the 86 adult-onset probands and 2 controls had nonsynonomous changes previously identified as disease causing, while none of these changes were found in the early-onset POAG probands. In the adult-onset families, the disease causing nonsynonomous SNPs did not segregate with the disease. These results indicate that DNA sequence variants in WDR36 do not significantly contribute to early-onset or adult-onset primary open angle glaucoma in this population. Further studies of patients with normal or low tension glaucoma may help define the role of WDR36 in this blinding disease.

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Evaluation of Complex Inheritance of Bardet-Biedl syndrome in a Puerto Rican BBS Cohort. *A.S. Cornier[1], D. Nishimura[2], A. Quintero-Del- Rio[1], C. Searby[2], E. Berg[2], G. Cox[4], S. Carlo[1], N. Ramirez[1], J. Acevedo[5], V. Franceschini[1], N. Arciniegas[1], V. Sheffield[2,3].* 1) Genetic Division, Ponce Sch Medicine, Ponce, PR; 2) Departemnt of Pediatrics, Univ of Iowa, Iowa City, IA; 3) Howard Hughes Medial Institution,Univ. of Iowa, Iowa City, IA; 4) Genetic Division Childrens's Hospital, Boston, MA; 5) Department of Nursigng, Univ. of Puerto Rico, Arecibo, PR.

Bardet-Biedl Syndrome (BBS) is a pleiotropic, autosomal recessive disorder characterized by retinal dystrophy, obesity, polydactyly, hypogenitalism and learning disabilities. BBS is genetically heterogenous with eight genes identified to date and evidence for additional loci. It has been suggested that in some cases, BBS may be inherited in a complex manner with three disease alleles (two at one locus and a third at a second locus) required for complete penetrance. The BBS1 gene was initially identified by studying a large Puerto Rican BBS cohort. Within this population, three distinct BBS1 mutations were found to segregate in different nuclear families. However, in two families, no BBS1 mutation could be found within the BBS1 coding sequence. We therefore decided to perform linkage studies on these families to determine if other BBS loci were involved. Genome-wide SNP genotyping indicated linkage to the BBS7 locus. Sequencing of BBS7 revealed a homozygous T211I mutation in all eight affected individuals from both of the non-BBS1 families. This mutation has been reported previously and T211 is highly conserved. Although the Puerto Rican population segregates mutations in both BBS1 and BBS7, inheritance of BBS in all individuals in the families studied is consistent with autosomal recessive inheritance.

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Evidence for linkage and association on chromosome 1q21 in Mexican families with familial combined hyperlipidemia. *A. Huertas-Vazquez[1, 2], C. Aguilar-Salinas[2], J. Lee[1], A. Lusis[1], R. Cantor[1], S. Canizales-Quinteros[2], T. Tusie-Luna[2], P. Pajukanta[1].* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Unidad de Biología Molecular y Medicina Genómica del Instituto de Investigaciones Biomédicas de la UNAM y del INCMNZS, Mexico City, Mexico.

Familial combined hyperlipidemia (FCHL) is a common genetically heterogeneous disorder, characterized by the presence of multiple lipoprotein phenotypes that increase the risk of premature coronary heart disease. A recent study of Finnish FCHL families reported an association between specific alleles of upstream transcription factor 1 (USF1) on chromosome 1q21 and the FCHL and high triglyceride (TG) traits. USF1 encodes a transcription factor known to regulate several genes of glucose and lipid metabolism, providing an excellent candidate gene for the complex FCHL phenotype. Previously seven extended multigenerational Mexican FCHL families have been linked to the chromosome 1q21 region for the FCHL and TG traits. We analyzed these seven and 17 additional extended Mexican FCHL families with 314 genotyped individuals, 144 of which were affected, for association with SNPs in the USF1 gene region. Three SNPs in the USF1 region (hCV1459766, rs3737787 and rs2073658) resulted in significant p-values (0.05-0.001) when tested for association with high TGs. To elucidate the potential role of USF1 as a causative gene for FCHL, probands of the four most strongly linked Mexican FCHL families and 4 normolipidemic spouses were sequenced for 11 exons and the exon-intron boundaries of USF1. Seven variants were identified, however none of these results in an amino acid change. The potential functional role of each associated variant is currently under evaluation with cross-species sequence comparisons and functional studies in relevant tissues to assess if any of these variants alter regulation of USF1 in cis. These Mexican data provide further evidence for the role of USF1 in FCHL.

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Evidence for linkage on chromosome 3q25-27 in a large autism extended pedigree. *H. Coon[1], N. Matsunami[2], J. Stevens[2], J. Miller[1], C. Pingree[1], L. Krasny[1], J. Lainhart[1], M.F. Leppert[2], W. McMahon[1].* 1) Neurodevelopmental Genetics Project, Dept Psychiatry, Univ Utah, Salt Lake City, UT; 2) Dept Human Genetics, Univ Utah, Salt Lake City, UT.

Though autism shows strong evidence for genetic etiology, specific genes have not yet been found. We tested for linkage in a candidate region on chromosome 3q25-27 first identified in Finnish autism families (Auranen et al., 2002). The peak in this previous study was at D3S3037 (183.9 cM). We tested this region in seven affected family members and 24 of their relatives from a single large extended Utah pedigree of Northern European ancestry. A total of 57 single nucleotide polymorphisms (SNPs) were analyzed from 165 cM to 200 cM. The maximum lod score was 3.40 (empirical p-value = 0.0004) across three SNPs from 184.6 - 184.9cM, close to the Finnish peak. We tested for alterations in a candidate gene in this region, the fragile X autosomal homolog, FXR1. No mutations were found in the coding sequence, exon-intron boundaries, or the promoter region of this gene.

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Association of TNF/LTA polymorphisms with Crohns disease in a Korean population. *K. Song[1], S. Lee[2], J. Lim[1], Y. Cho[3], S. Yang[3].* 1) Dept of Biochemistry & Molecular Biology, Univ of Ulsan College of Medicine, Seoul, Korea; 2) Dept of Biotechnology, College of Agriculture & Life Sciences, Chonnam National University, Gwangju, Korea; 3) Dept of Internal Medicine, Univ of Ulsan College of Medicine, Seoul, Korea.

The tumor necrosis factor-a (TNF) and lymphotoxin-a (LTA) are proinflammatory and immunoregulatory cytokines. Promoter polymorphisms of the TNF have been shown to affect TNF production and to be associated with Crohns disease, however, the actual alleles associated with the disease were variable among populations. Five TNF promoter polymorphisms (-1031, -863, -857, -308, and -238) and two LTA polymorphisms (intron 1 and Thr60Asn) were analyzed in 288 patients with Crohns disease and 399 unrelated healthy controls. Carriers of an individual polymorphism of TNF at -1031C, -863A, and -857T, showed statistically significant association with Crohns disease (adjusted OR = 1.51, 95 % CI = 1.08-2.10, P = 0.016; adjusted OR = 1.44, 95 % CI = 1.02-2.02, P = 0.038; adjusted OR = 1.60, 95 % CI = 1.13-2.27, P = 0.008), respectively. Following the haplotype analysis, carriers of the haplotype consisted of the -1031C, -863A, and -857C alleles showed statistically significant association with Crohns disease (adjusted OR = 1.58, 95 % CI = 1.10-2.23, P = 0.014). Our results are similar to those of two Japanese case-control studies, however different from Caucasian data. The carriers of the polymorphism at Thr60Asn of LTA had age/sex-adjusted odds ration of 0.61 (95 % CI = 0.43-0.87, P = 0.007), showing protective effects on Crohns disease. Our data support the hypothesis that the TNF/LTA genotypes play an important role in the pathogenesis of Crohns disease.

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MMP7 promoter polymorphisms -181A/G and -153C/T are not associated with chronic pancreatitis in the United States. *J. Lamb[1,5], Y. Zhang[2], J.J. Martinson[3], L. Wang[2], B.D. Elinoff[1,5], D.C. Whitcomb[1,3,4,5].* 1) Dept Medicine, Div Gastro, Hep & Nutrition; 2) Div PACC; 3) Dept Human Genetics; 4) Dept Cell Biol & Phys, Univ Pittsburgh; 5) UPCI Hillman Cancer Ctr, Pittsburgh,PA.

Chronic pancreatitis (CP) is an inflammatory disease of progressive fibrosis and calcification leading to destruction of the pancreas. Smoking is a strong risk factor for CP and appears to accelerate progression of pancreatic fibrosis and insufficiency. Clinical imaging and functional tests can only detect CP at an advanced stage when functional recovery is minimal. An understanding of the etiology of the disease will allow earlier identification and intervention for those at risk.

Matrix metalloproteases (MMPs) are a family of proteases involved in extracellular matrix homeostasis and pathologic remodeling. MMP7 is associated with idiopathic pulmonary fibrosis (IPF). Pathologically similar to CP, IPF is a disease also characterized by progressive fibrosis and tissue distruction. Two functional polymorphisms have been identified in the MMP7 promoter at -181 (-181 A/G) and -153 (-153 C/T). Moreover it has recently been shown that a -181 A allele increases susceptibility to IPF in smokers.

We have investigated whether functional polymorphisms in the MMP7 promoter are associated with CP in smokers and non-smokers. Ninety-one CP, 49 recurrent acute pancreatitis (RAP) and 206 healthy control (HC) samples were genotyped by PCR RFLP analysis. Haplotypes were determined and analyzed using PHASE software. No significant differences were found between CP and RAP (which is not associated with fibrosis), $p = 0.9$; or CP and HC datasets, $p = 0.54$. Comparison of haplotypes of CP smokers and HC smokers similarly gave a non-significant result $p = 0.47$. Considering only the -181A/G polymorphism a ^{2}test showed no association of the A allele in CP smokers, $p = 0.84$. We therefore conclude that there is no association between the MMP7 -181G/A and -153C/T promoter polymorphims and chronic pancreatitis. Further, unlike IPF, the -181 A allele does not appear to increase susceptibility to CP in smokers.

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Interferon-inducible gene family *HIN200* and lupus susceptibility. *L.J. Flinn[1,3], D. Xu[2], M. Lee[2], M-C. King[1,2].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Medical Genetics, Medicine, University of Washington, Seattle, WA; 3) Molecular and Cellular Biology Program, University of Washington, Seattle, WA.

Systemic Lupus Erythematosus (SLE) is a complex disease influenced by genetic background and environment. In order to identify genes that contribute to lupus susceptibility, we chose to examine a candidate gene family, the *HIN200* genes, clustered in a 250-kb region within 1q23. These genes are homologous to the *Ifi200* genes in mouse, implicated in the *Nba2* mouse model of SLE. The human gene family consists of four genes: *MNDA*, *IFIX* or *PYHIN*, *IFI16*, and *AIM2*, all similar in protein structure. We used genomic sequencing to detect a frameshift mutation in the first coding exon of *AIM2* in one SLE case, resulting in protein truncation. This mutation was not seen in 500 additional SLE cases and controls. We are currently sequencing the coding regions and flanking UTRs of all four genes in a series of 69 SLE cases and matched controls, and have not yet detected any other obviously pathogenic mutations. We are searching for variants altering gene function in other ways, in addition to those that affect protein sequence. We use linkage disequilibrium (LD) analysis to determine haplotype blocks with this region, and then use haplotype-tag SNPs (htSNPs) to further identify disease-associated alleles. Variants with potential regulatory effects will be in LD with htSNPs, and haplotypes associated with disease examined more closely. This method also tests for association of the entire gene cluster with disease. We have identified four major haplotype blocks; the largest, at nearly 80 kb, harbors *MNDA* and is defined by 14 SNPs (MAF0.10). The most common haplotype in this region occurred at decreased frequency in SLE cases (p0.005), and cases had a corresponding increase in frequency of rarer haplotypes. Our next steps will be to examine variation in this block, by sequencing potential regulatory domains and genotyping more SNPs. We hypothesize that a regulatory variant in this region may influence expression levels, which may predispose certain individuals to lupus susceptibility.

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Association analysis of 47 candidate genes in 200 attention-deficit/hyperactivity disorder(ADHD) trios. *M.N. Ogdie[1], J. Ishii[1], S.L. Smalley[2], S.F. Nelson[1].* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Center for Neurobehavioral Genetics, UCLA Neuropsychiatric Institute, David Geffen School of Medicine at UCLA, Los Angeles, California 90024.

Attention-deficit/hyperactivity disorder (ADHD) is a pervasive neurobehavioral disorder characterized by childhood onset and impairment in multiple settings. ADHD affects ~5% of children and adolescents, and ~3% of adults. We have conducted transmission-disequilibrium analyses of 47 candidate genes in 200 ADHD trios. Over 400 SNP markers were selected and genotyped in a set of genes comprising both functional candidates and positional candidates located in previously linked regions. Transmission-disequilibrium tests (TdT) of singleton SNPs and haplotypes in two genes, prolactin-inducible protein (PIP) and the serotonin transporter (5-HTT), yielded evidence of association to ADHD. Singleton SNPs in PIP present two-fold over-transmission to cases and nominally significant evidence of association (P = 0.0002). Additionally, a haplotype in PIP is four-fold over-transmitted to affected individuals (P = 0.0004). The serotonin transporter, located within the previously linked region on 17q11, presents nominally significant association (P = 0.0035). In the current study, we report an exhaustive analysis of all 47 candidate genes and discuss putative associations to ADHD.

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Investigation of DGCR8 and the miRNA pathway in schizophrenia. *S.E. Bruse, M.A. Azaro, I.V. Tereshchenko, L.A. Goff, R.P. Hart, L.M. Brzustowicz.* Department of Genetics, Rutgers University, Piscataway, NJ.

Linkage analyses have repeatedly identified the 22pter-q12.3 chromosomal region as linked to schizophrenia. DGCR8 is a positional candidate given its location at 22q11.21. It was recently discovered that DGCR8 functions in the microRNA (miRNA) pathway. miRNAs are important in regulating protein expression and are abundant in mammalian neurons. Perturbation of these regulatory pathways could conceivably contribute to the etiology of schizophrenia. We wish to determine whether DGCR8 is associated with schizophrenia using family-based genetic analysis. In addition, we are investigating the expression patterns of miRNAs in post-mortem brain samples of schizophrenic patients. 22q11.21 contains other strong candidate genes, and a simultaneous investigation of DGCR8, COMT, and ZDHHC8 was performed. A SNP tagging strategy was used which utilized the r2 measure of LD to select one tag SNP from each of 26 LD bins spanning these three genes. Thus, 26 SNPs were genotyped in a set of 37 extended pedigrees of Caucasian descent. Single-marker analysis was performed using the Pedigree Disequilibrium Test. At least one SNP in all three genes showed nominal evidence for association ($p<0.05$). SNP rs2255497, located within the 3 UTR of DGCR8, showed the strongest association with schizophrenia ($p<0.0045$), and was the only SNP with significant association after correction for multiple testing. Interestingly, this SNP is located within a predicted conserved miRNA binding site (has-mir-221). Ongoing studies include an attempt to replicate this finding in an independent Caucasian sample of 24 extended pedigrees. We have also begun an miRNA microarray study of post-mortem schizophrenic brain samples using a microarray containing 218 known human miRNAs (mirMAX, Rutgers University). A pilot study examined post-mortem samples from the medial temporal lobe of three schizophrenics compared to three controls. Though some striking individual expression differences were observed, no miRNAs showed significant expression differences after correction for multiple testing. Ongoing studies include miRNA microarray analysis of multiple brain regions in a larger set of samples.

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Further evidence in support of allelic and haplotypic association of GABAA receptors and alcohol dependence in an Irish sample. *G. Kalsi[1], P-H. Kuo[1], J. Vittum[1], P.F. Sullivan[2], E.J.C.G. van den Oord[1], D.G. Patterson[3], D. Walsh[4], K.S. Kendler[1], C.A. Prescott[1], B.P. Riley[1].* 1) Molecular Genetics Lab, Virginia Commonwealth Univ., Richmond, VA; 2) Dept of Genetics, University of North Carolina, Chapel Hill, North Carolina; 3) Shaftsbury Square Hospital, Belfast, Northern Ireland, UK; 4) Health Research Board, Dublin 2, Ireland.

Animal and human studies suggest that GABAA receptors play a role in the neurochemical pathways involved in alcohol use, abuse and dependence. GABAA receptor subunits have been tested in several association studies, which have supported their role as candidates for alcohol dependence. The current case-control study was conducted in a subset of the larger Irish Affected Sib Pair Study of Alcohol Dependence (Prescott et al, 2005). 328 unrelated cases and 328 controls of matched ethnicity were genotyped with 21 SNPs localized to GABRG1, GABRA2, GABRA4 and GABRB1 subunits on chromosome 4p12. The results were analyzed using the WHAP program. Four of the nine GABRA2 SNPs tested in our sample revealed allelic association with alcohol dependence. Furthermore, one of the SNPs for GABRB1 produced a highly significant association (p=0.000012). Computing all nine GABRA2 SNPs for haplotype analyses using WHAP produced a disease associated haplotype with a p-value of p=0.02. Thus, our result replicates previously reported studies. Additional joint haplotype analyses of the nine 2 subunit SNPs and the most significantly associated 1 subunit SNP produced an enhanced disease association, with p value of 0.000067. This result may be indicative of an epistatic effect which could have functional implications, however further tests are required to verify whether a true interaction does exist. Prescott et al (2005) Alcohol Clin Exp Res. 29(3):417-29 This work was supported by NIH grant AA-11408.

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A detailed study of the serotonin pathway in autism. *B.M. Anderson[1], L. Jiang[1], N. Schnetz-Boutaud[1], H.H. Wright[3], R.K. Abramson[3], M.L. Cuccaro[2], J.R. Gilbert[2], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine, Duke University Medical Center, Durham, NC; 3) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Classic autism is characterized as one of the Pervasive Developmental Disorders (PDDs) of childhood, a spectrum of often severe behavioral and cognitive disturbances of childhood development. The high heritability in autism has driven multiple efforts to identify susceptibility genes, but to no avail. Numerous studies have suggested that deficits in the peripheral and central metabolism of serotonin may play a role in the pathophysiology of autism. We hypothesize that multiple variations in the serotonergic pathway genes are involved in autism.

We assembled a dataset collected through the collaborative efforts of Vanderbilt and Duke Universities. Our sample includes 284 total autism families consisting of 155 parent-child trios and 129 multiplex families, a screening subset of our overall dataset of 333 trios and 158 multiplex families. We prioritized our initial work to include 10 prominent serotonin pathway candidate genes for detailed study within our autism families.

Sixty evenly spaced SNPs were genotyped in our candidate genes: HTR1A, DDC, TPH1, TPH2, SLC6A4, ITGB3, AANAT, FLJ22341, MAOA and MAOB. Parametric two-point analyses identified peak LOD scores > 2.0 for the subset of families containing only affected males within AANAT, LOD=2.63, and SLC6A4, LOD=2.04 for the complete Vanderbilt subset. Another marker within SLC6A4, demonstrated a parametric two-point LOD of 2.61 in the Vanderbilt male-affected-only families and a highly significant association in the Vanderbilt trio subset(0.006). However, there was little evidence of linkage or association in the Duke subset suggesting that underlying heterogeneity remains. These results warrant further investigation of the serotonergic pathway including a specific examination of gene x gene interactions (underway).

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Investigation OF *LAMB1* as an Autism candidate gene. *D.A. Skaar[1], J.M. Jaworski[1], J.L. Benton[1], E.R. Martin[1], H.H. Wright[3], R.K. Abramson[3], J.L. Haines[2], M.L. Cuccaro[1], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Center for Human Genetics Research, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 3) University of South Carolina, Columbia, SC.

Multiple studies indicate an area on chromosome 7q as potentially contributing to autism susceptibility. Several microsatellite markers on 7q, d7s1813 (102.82cM), d7s821 (109.1cM), d7s477 (111.79cM), and d7s496 (119cM), have shown significant linkage. In addition, haplotype and recombination analysis has identified the region 119.8-122.5cM as being predominantly shared among families with autistic individuals. These indications, combined with the candidate gene *RELN* also located in this area of 7q, led us to perform a fine-mapping study of this region (111.3-121.4cM/98.8Mb-108.5Mb) with SNPs spaced approximately 100kb apart. Three areas with multiple association peaks were identified with this screen. One of these significant regions (~107-108Mb) contains the *NRCAM* and *LAMB1*, which have been previously examined as candidate genes. Multiple SNPs in these genes have been tested by two different groups, with both groups finding significant associations in *LAMB1* SNPs. One group found significance for a missense mutation in exon 30 that changes a conserved isoleucine (I1547) to threonine, while the other group identified a significant SNP in intron 3.

We therefore examined the *LAMB1* SNPs tested by the other groups in our dataset. Twelve SNPs were typed on 501 Caucasian families. No significant association results were seen for any of these SNPs for this dataset. Several SNPs showed marginally significant linkage scores (LOD1.0), but neither of the SNPs that were found associated in the previous studies had linkage approaching significance.

This lack of replication indicates that *LAMB1* is probably not contributing to Autism susceptibility in these data. However, our association results in this region of 7q continue to support the presence of a susceptibility factor.

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Genetic polymorphisms in catechol O-methyltransferase gene (COMT) affect acute post-surgical pain in humans.
H. Kim, D. Mittal, J. Brahim, J. Rowan, R. Dionne. Dept PNMB, NIDCR/NIH, Bethesda, MD.

Candidate gene studies on the basis of biological hypotheses have been a practical approach to identify relevant genetic variation in complex traits such as pain. Based on their roles in pain pathways, we have examined the effects of variations of loci in the human catechol O- methyl transferase gene (*COMT*) and cyclooxygenase 1 and 2 genes, also known as prostaglandin G/H synthase genes (*PTGSs*), on acute clinical pain responses in humans. In the responses to clinically induced acute pain, haploblocks from COMT showed significant association with maximum post-operative pain ratings. Further analysis revealed that homozygous SNP7 (rs165722) T/T patients in the *COMT* haploblock 2 (HB2) showed significantly lower pain ratings than C/C homozygous patients (t-test, $p = 0.031$) while no significant association was found between clinically induced acute pain responses and haploblocks in *PTGS1* and *PTGS2*. However, the association between *COMT* genetic variations and pain sensitivity in our study differ from previous studies with small sample sizes, population stratification and artificial pain phenotype from combining different types of pain stimuli. The association between *COMT* SNP7 and persistence in psychological temperament suggests interactions between genetically influenced psychological trait and acute clinical pain perception.

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Association of a SNP in the *PTPN22* gene with multiple autoimmune diseases. *S. Onengut-Gumuscu[1], D.K. McCurdy[2], C. Pihoker[3], J.H. Buckner[1], C. Greenbaum[1], P. Concannon[1].* 1) Benaroya Research Institute, Seattle, WA; 2) Division of Rheumatology, Mattel Children Hospital, Los Angeles, CA; 3) Children's Hospital and Regional Medical Center, Seattle, WA.

The *PTPN22* gene at chromosome 1p13 encodes a 110 kDa lymphoid specific tyrosine phosphatase, Lyp, that negatively regulates the response of T cells to antigen stimulation. A coding SNP in *PTPN22* (1858T) (R620W) has been reported to be a general risk allele for autoimmunity possibly due to its effects on the interaction of Lyp with the Csk kinase during T cell activation. To evaluate the role of variation at the *PTPN22* 1858C/T SNP in autoimmunity we have genotyped independent populations with adult rheumatoid arthritis (RA) (N = 68), pauci-articular juvenile rheumatoid arthritis (PJRA) (N = 79), and type 1 diabetes (T1D) (N = 244) and compared allele frequencies in these cases to a single control population (N = 167). The frequency of the T allele in controls was 0.086 (95% CI = 0.0590.12). The RA population had a significantly higher T allele frequency when compared to controls (0.169, 95% CI = 0.110.243) (P = 0.008). In the PJRA population, the frequency of the T allele was increased (0.127, 95% CI = 0.0790.189), but was not significantly different from controls (P = 0.1). The frequency of the T allele among T1D cases was significantly elevated relative to controls (0.158, 95% CI = 0.1260.193) (P = 0.001). We confirmed the association with T1D by genotyping 406 multiplex T1D families. There was significant evidence of association between the 1858T allele and T1D by pedigree disequilibrium testing ($P = 2.5 \times 10^{-5}$). T1D families from this analysis in which affected siblings share alleles identical by descent at microsatellite markers flanking *PTPN22* are being screened for additional coding variants that may contribute to autoimmunity.

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The importance of non - invasive genetic analysis of Col4a5, Col4a4 and Col4a3 genes in the initial diagnostics of Alport syndrome and familial benign hematuria. *D. Glavac[1], M. Ravnik-Glavac[1], M. Slajpah[1], P. Furlan[2], A. Meglic[3].* 1) Department of Molecular Genetics, Institute of Pathology, Medical Faculty, Korytkova 2, 1000 Ljubljana, Slovenia; 2) Department of Nephrology, General Hospital Novo mesto, Smihelska c. 1, 8000 Novo mesto, Slovenia; 3) Pediatric Nephrology Department, Childrens Hospital, University Medical Center Ljubljana, Stare Pravde 4, 1000 Ljubljana, Slovenia.

Alport syndrome is a hereditary disease, characterized by progressive hematuric nephritis with structural changes of glomerular basement membrane, frequently associated with sensorineural hearing loss. The distinction between Alport syndrome and familial benign hematuria is sometime difficult in young patients, sporadic cases and small families. Major changes in glomerular basement membrane are associated with mutations in Col4a5 gene which encodes for a5 collagen chain. However the disease was also associated with Col4a3 and Col4a4 genes. DNA was extracted from peripheral blood lymphocytes of five children (three boys, two girls) aged 10 months to four years. Col4a3, Col4a4 and Col4a5 genes were amplified in PCR and analyzed using non-isotopic high resolution single stranded conformation analysis (SSCA) and sequenced on ABI PRISM 310 Genetic Analyzer. Clinical data were obtained from clinician who performed precise urine tests, hearing and ocular examinations. Three novel mutations, G198E, G3189D and G669R, were detected in Col4a5 gene and one new mutation 3145del5b in Col4a3 gene. On the basis of the results of our study and the existing national register of Alport syndrome patients, we demonstrated that non-invasive methods such as the genetic analysis of collagen genes, particularly in the case of young patients with undefined clinical features, may be of great importance and could diminish the need for invasive skin and renal biopsy. Some patients affected with familial benign hematuria are carriers for autosomal recessive Alport syndrome. Our study showed the importance of molecular genetic data for the purpose of providing quick and precise diagnoses to affected family members and their offspring.

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Prediction of radiation morbidity from polymorphisms in candidate genes among prostate cancer patients treated with carbon ion therapy. *M. Iwakawa[1], T. Suga[1], S. Noda[1], H. Tsuji[2], Y. Ohtsuka[1], M. Kohda[1], M. Sagara[1], A. Ishikawa[1], H. Suzuki[1], J. Mizoe[2], H. Tsujii[2], T. Imai[1].* 1) Frontier Research center, National Institute of Radiological Sciences, Chiba, Chiba, Japan; 2) Research Center for Charged Particle Therapy, National Institute od Radiological Sciences, Chiba, Chiba, Japan.

Heavy ion beams possess high linear energy transfer components and a prominent Bragg peak in the human body. These properties promote higher relative biological effectiveness and a favorable dose distribution. Some patients, however, develop adverse effects in the rectum and /or bladder/urethra. Using DNA samples collected from 95 Japanese prostate cancer patients who underwent radiotherapy with the Heavy Ion Medical Accelerator in Chiba, 905 SNPs were genotyped from 127 candidate genes for radiation susceptibility. These genes were selected from our previous gene expression analyses of cultured human cell lines and mouse strains that had exhibited variable radiosensitivities. The SNPs in the candidate genes were selected from jSNP and dbSNP databases and their allele frequencies were examined using 133 healthy controls. The acute and late radiation morbidities were scored using Late Effects of Normal Tissues-subjective, objective, management, and analytic criteria. Significant differences were observed of genotype frequencies for SNPs in candidate gene loci. Area under the curve of the Receiver Operating Characteristic Curve (ROC) was estimated for each SNP marker for radiation morbidity to construct a predictive score. The top 7 markers were used in constructing predictive scores for dysuria within 3 months, and the top 5 markers at 6 months after starting radiotherapy. The top 10 markers were used in constructing predictive scores for polyuria within 3 months, and the top 11 markers at 6 months after starting radiotherapy. The distribution of ten predictive scores according to response values showed the potential discriminating power of these analyses.

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Association Genome Scanning for Addiction Vulnerability: Convergent Results Using 98,398 SNPs and Four Samples. *G. Uhl[1], Q. Liu[1], T. Drgon[1], C. Johnson[1], D. Walther[1], I. Sora[2], H. Ujiki[2], T. Inada[2], M. Yamada[2], N. Iwata[2], M. Iyo[2], I. Ozaki[2], Y. Sekine[2], H. Yoshimoto[2], M. Harano[2], T. Komiyama[2].* 1) Molec Neurobiology, NIDA/NIH & JHUSM, Baltimore, MD; 2) Japanese Genetics Initiative on Drug Abuse (JGIDA).

About half of human vulnerability to substance abuse and dependence comes from polygenic allelic variants, including common alelic variants that are likely to contribute to vulnerability to many addictive substances. To identify the chromosomal regions likely to contain such variants, we have validated and used pooled microarray-based association genome scanning to compare allelic frequencies at 98,398 autosomal SNP markers in four samples European American and African American polysubstance abusers/controls recruited in Baltimore, European-American alcohol dependent and control individuals from a multicenter US sample, and Japanese methamphetamine abusers/controls (ca 160 million person/ genotypes determined in quadruplicate). SNPs that display 1) the largest allelic frequency differences between substance abusers and controls and 2) the largest nominal statistical significance for these differences are found much more closely clustered in several chromosomal regions than expected by chance. Regions that contain these clustered, positive SNPs in multiple samples contain haplotypes that include interesting genes involved in cellular information processing, transcriptional and translational/posttranslational regulation, other interesting neuronal functions. These results, and other ongoing efforts to elucidate the molecular genetic underpinnings of human addictions, add additional powerful suport to the idea that common allelic variants contribute to individual differences in vulnerabilities to human addictions in individuals from a number of different racial/ethnic groups.

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Sib-pair analysis in Brazilian families with non syndromic cleft lip with or without cleft palate. *F. Poerner[1,2,3], M. Bicalho[2], T. Hiekkalinna[4], M. Passos-Bueno[1].* 1) Biology, USP, São Paulo, Sao Pulo, Brazil; 2) Genetics, Biology, UFPR, Curitiba, Brazil; 3) CAIF, Curitiba, Brazil; 4) National Public Health Institute, Department of Molecular Medicine,Helsinki, Finland.

Cleft lip with or without cleft palate (CL/P) is one of the most common congenital malformations, occurring in 1:200 to 1:2000 live births, depending on the ethnic group. It is an heterogenous condition and the multifatorial model of inheritance is the most acceptable. To date, there is still no consensus which are the candidate genes involved with the predisposition of this condition. Therefore, our major aim is to identify susceptibility loci for non syndromic CL/P in the Brazilian population. For this purpose, we have applied the sib pair approach using microsatellite markers of 14 chromosomes (1, 2, 4, 6, 8, 11, 12, 13, 14, 16, 17, 19, 22 and X) that were previously found associated with CL/P through others methods of analysis (Carinci et al, J. Craniof. Surg., v.14, n.2, 2003). We have so far included 50 affected sib-pairs with nonsyndromic CL/P from 44 families which were ascertained in CAIF - Center for Integral Care to Cleft Lip and Palate Patient, Curitiba, PR, Centrinho, Joinville, SC and Center of Studies in Human Genome, SP. A total of 232 microssatelites markers have been analyzed and the results were analyzed using the GeneHunter software. The major evidence of linkage was found in chromosome 1 region between markers D1S2868 e D1S484 (max lod score of 3.24). The present data will also be analyzed with the software Pseudomarker. Other regions with smaller values of lod score will be discussed.

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Genome-wide association for Atypical Antipsychotic Treatment Emergent Weight Gain and Replication in an Obesity Cohort. *S. Kirkwood[1], R.D. Hockett[1], N. Mukhopadhyay[1], D.J. Fu[1], D. Ballinger[2], R.A. Price[3], Y. Yue[1], L. Gelbert[1], S. Li[1], D. Cox[2], A. Breier[1].* 1) Res Tech/Experimental Med, Eli Lilly & Co, Indianapolis, IN; 2) Perlegen Sciences, Inc, Mountain View, CA; 3) University of Pennsylvania, Philadelphia, PA.

Treatment-emergent weight gain observed with atypical antipsychotic therapy continues to be a clinical concern with genetic factors likely playing a role. The genetic contribution to weight gain has been investigated using candidate gene methodology. Although significant associations with candidate genes such as the Serotonin 5-HT2c Receptor Gene have been reported, negative results have also been. The lack of consistent findings has led to uncertainty as to the significance of reported associations. Therefore, we completed a genome scan followed by a replication in a cohort of individuals selected for obesity. Using a cohort of adult patients diagnosed with schizophrenia, schizoaffective, or schizophreniform disorder who had taken oral olanzapine for a minimum of six months, cases and controls were chosen from the 20% tails of the weight-gain distribution, weight gainers (n=255) and non-weight gainers (n=258) as measured by change in body mass index. A genome-wide association study using the Perlegen Sciences platform was performed using pooling in phase I and then individual genotyping in phase II for all 513 individuals for 30,000 SNPs. Association analyses between the 30,000 SNPs and weight-gain phenotype were completed using Fishers exact test. Following the genome scan, an algorithm was developed using linkage disequilibrium map information and the association results from the treatment emergent weight gain scan (SNP significance level, frequency, and clustering information) to select SNPs for follow up (n=3000). Using a cohort of more than 340 parent child trios selected for an obese proband, these SNPs were then associated with obesity using transmission disequilibrium methodology.The SNPs (n=311 at p=0.001) and genes identified as significantly different in the first cohort between weight gainers and nongainers and those that replicated in the obesity cohort will be presented.

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Analysis of a QTL for diabetes related traits identified through a genome scan in large extended pedigrees. *J. Jowett[1, 5], J. Curran[4], K. Shields[1], J. Wang[1], S. Dennis[1], R. Kotea[2], S. Kowlessur[3], P. Chitson[3], P. Zimmet[1], J. Blangero[4, 5].* 1) Genetics Research, Intl Diabetes Inst, Melbourne, Australia; 2) SSR Centre, Mauritius; 3) Ministry of Health, Mauritius; 4) Southwest Foundation for Biomedical Research, San Antonio, TX; 5) ChemGenex Pharmaceuticals, Grovedale, Australia.

The prevalence of Type 2 diabetes and obesity continues to increase in both developed and developing countries presenting major public health issues impacting a wide variety of social and economic measures. Substantial evidence supports a major role of genetics in development of the disease together with modifying effects of the environment. To improve our understanding of the molecular mechanisms underpinning the metabolic abnormalities associated with disease development, we conducted a genome-wide scan to identify chromosomal regions likely to harbour disease-influencing genes. Using large multigenerational pedigrees to improve statistical power of susceptibility gene detection/localization we undertook a 10 cM genome scan on a cohort of 450 individuals in 20 families. We identified two quantitative trait loci (QTL) for fasting plasma glucose and uric acid with significant lod scores. To assist with prioritisation of candidate genes, we developed a data-mining software program that interrogates online databases for information relating each gene under the peak to the trait phenotype. To begin dissection of the QTL for fasting plasma glucose at 12q24, we selected SNPs from within and juxtaposing the top ranked genes and from intergenic regions showing conservation among mammals. Preliminary association analysis of SNPs typed in a set of founders identified 30 associated variants in 15 genes. The overall best signals (p-value = 0.0000192) were seen in novel genes that are now being assessed in the total sample.

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A genome-wide association study in sporadic ALS using DNA microarrays. *C.L. Simpson[1], V. Hansen[1], J. Powell[2], I. Fogh[1], M. Turner[1], C. Shaw[1], J. Knight[3], P. Sham[3], P. Leigh[1], A. Al-Chalabi[1].* 1) Dept Neurology, Inst Psychiatry, London, United Kingdom; 2) Dept Neuroscience,Inst Psychiatry, London, United Kingdom; 3) MRC SGDP Centre, Inst Psychiatry, London, United Kingdom.

Most cases of ALS occur sporadically, but twin studies suggest a genetic contribution of up to 85%. Previous association studies using candidate genes have yielded few positive, reproducible associations. DNA microarrays allow the genotyping of larger numbers of SNPs than is feasible with microsatellites and combining this approach with DNA pooling provides a quick and efficient method of performing a genome-wide association study. Our objective was to perform a genome-wide screen for susceptibility and phenotype modifier genes for sporadic amyotrophic lateral sclerosis (ALS), using DNA pools genotyped on Affymetrix GeneChip microarrays. We have previously analysed DNA pools with the 10K microarrays, and here present the results of this technique using the denser Affymetrix GeneChip 100K Mapping Set.

Pools were constructed of 300 cases and 300 controls matched for sex and age withing 1 year. 100 individual genotypes were used to calculate data correction values. Each pool was hybridised to the microarrays in 6 replicates and mean values used for analysis. 99,776 SNPs were analysable and had correction factors available. Using a modified chi square which allows for measurement and sampling error, 11,895 markers were significant at $P < 0.05$, 42 were significant at the Bonferroni corrected $P < 5 \times 10^{-7}$. The largest effect sizes were seen for SNPs on chromosome X (OR = 12, $P < 10^{-8}$) and chromosome 13 (OR = 8.3, $P = 10^{-15}$).

DNA microarrays provide the high-throughput technology which genome-wide association studies require. This work shows that genotyping DNA pools on microarrays is a useful method for first-pass screening which has quickly and efficiently identified regions associated with ALS to target with fine-mapping studies.

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Mitochondrial Genotypes Associated with Healthy Aging. *T.A. Donlon[1,2,3,4], J. Grove[2,3,4], B.J. Wilcox[2,3,5], K. Yano[2,3], R. Chen[2], B.L. Rodriguez[2,3,5], K.H. Masaki[2,3,5], C. Ceria[2,6], J.D. Curb[2,3,5].* 1) Queen's Genetics Laboratories, The Queen's Medical Center, Honolulu, HI; 2) Pacific Health Research Institute, Honolulu, Hawaii; 3) Honolulu Heart Program, Kuakini Medical Center, Hawaii; 4) Cancer Research Center of Hawaii, University of Hawaii, Honolulu; 5) Department of Geriatric Medicine and Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu; 6) Department of Nursing, School of Nursing & Dental Hygiene, University of Hawaii, Honolulu.

As our society ages it becomes imperative to identify causes of morbidity and decline in order to improve the quality of late life. The mitochondria plays a major role in many age-related chronic diseases and may play an important role in healthy aging and longevity. Healthy aging was measured using hand grip strength at two separate exams in an elderly male Japanese population and shown to be associated with common polymorphisms in the genes for three mitochondrial proteins, one mitochondrial- and two nuclear-encoded; NAD Dehydrogenase, subunit 2 (NAD2 C1578A), Uncoupling Protein 1 (UCP1; G/T in 5 UTR), and Uncoupling Protein 2 (UCP2; V55A). These results show that common variation in mitochondrial genes can have a significant effect on healthy aging, even in late life and identify an important physiologic pathway for future investigations.

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Haplotype-based sequencing methodology to identify strongly associated SNPs in regions with strong LD, applied to EBV positive Hodgkin Lymphoma. *M. Niens[1], A. van den Berg[2], A. Diepstra[2], I. Nolte[3], G. van der Steege[3], S. Poppema[2], G.J. te Meerman[1].* 1) Department of Medical Genetics; 2) Department of Pathology; 3) Department of Medical Biology, University Medical Center Groningen, Groningen, Netherlands.

Various studies have indicated that the human leukocyte antigen (HLA) region is associated with Hodgkin Lymphoma (HL). We recently demonstrated association of the HLA class I region with Epstein-Barr virus (EBV) positive HL cases. The positively associated haplotype was characterized by alleles 126 and 284 of the consecutive microsatellite markers D6S265 and D6S510 while the negatively associated haplotype was characterized by alleles 130 and 302. Further fine mapping of this region is necessary to identify the causal SNP(s). To select the most promising candidate SNPs for screening the total study population, known SNPs were sequenced in an individual homozygous for the positively associated haplotype and an individual homozygous for the negatively associated haplotype. Four SNPs (rs2523972, rs4713276, rs2256543, and rs6457110) that were selected on the basis of displaying different alleles in these two individuals where analysed in the total study population and revealed a much stronger association than the microsatellite markers (-log p difference = 2). The associated region was delimited from 310 kb in the original study to a region comprising less than 60 kb in the present study. Of the genes mapping to this candidate region, HLA-A represents the most interesting target because of its consistent expression in EBV positive HL cases and its ability to present EBV derived peptides to cytotoxic T cells. We demonstrated that sequencing homozygous individuals selected for positively and negatively associated haplotypes is an efficient way to identify candidate SNPs.

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Identifying genes for optimal brain aging: translational gene mapping of complex phenotypes. *B. Wilmot[1], S. McWeeney[2], R. Nixon[3], J. Laut[4], J. Kaye[4], P. Kramer[1,4].* 1) Dept Mol. & Med. Genetics, Oregon Health & Sci Univ, Portland, OR; 2) Div. of Biostatistics, Dept of Public Health and Prev. Medicine, Oregon Health & Sci Univ, Portland, OR; 3) Dept of Pathology, Oregon Health & Sci Univ, Portland, OR; 4) Dept of Neurology, Oregon Health & Sci Univ, Portland, OR.

Healthy brain aging is a complex phenotype subject to both genetic and environmental influences. Natural variation across individuals in the ability to sustain cognitive function in the presence of brain injury is known as cognitive reserve. Based on the hypothesis that cognitively healthy individuals carrying a severe neuropathologic burden may be utilizing cellular pathways in a different manner, we designed a case/control study in which the controls were classified into defined phenotypic groups based on severity of neuropathology (Braak stage) and were compared to patients with Alzheimers disease (AD), the most common cause of dementia in the elderly. Global gene expression profiling was conducted on nine cognitively intact individuals and five AD patients in postmortem frontal cortex tissue. Of the 4556 transcripts that were differentially expressed (q-value < 0.05) between control subjects and AD subjects, 58% were downregulated in AD subjects. 487 genes were located in previously identified linkage regions. 1655 genes in the list of differential expression candidates were statistically overrepresented in Gene Ontology (G0) categories for biological process when compared to the expected proportion based on the distribution of GO categories on the gene array. Genes implicated in cognitive reserve were identified using trend analysis across all groups. Of the 82 transcripts identified, 51 transcripts were upregulated in controls with Braak stage V/VI as compared to AD subjects. Comparison of the differentially expressed genes involved in AD with those implicated in cognitive reserve revealed only 5 genes common to both suggesting the presence of unique cellular processes in brain tissue of cognitively healthy individuals with severe neuropathology. Multiple Attribute Decision Making theory was used to create a priority score to identify genes for further study.

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Polymorphism 5 of the Leptin Gene Results in Sex-Specific Trends in Birth Weight and Cord Leptin Levels. *R.M. Adkins[1], C.K. Klauser[2], J. Fain[3], J. Magann[4], J. Morrison[2].* 1) Le Bonheur Child Med Ctr, Univ Tennessee-Hlth Sci Ctr, Memphis, TN; 2) Department of Obstetrics and Gynecology, Univ. MS, Jackson, MS; 3) Department of Molecular Sciences, Univ. TN Hlth Sci Ctr., Memphis, TN; 4) Department of Obstetrics and Gynecology, Naval Medical Center - Portsmouth, Portsmouth, VA.

Objectives: Leptin levels are positively correlated with adult BMI and birth weight, with female serum levels nearly twice those of males. Postnatally, SNPs in the leptin gene have been shown to exhibit sex-specific associations with BMI and circulating leptin levels. These relationships, as they affect fetal birth weight, remain undefined. The purpose of this study is to test the association between leptin polymorphism and both birth weight and cord leptin levels.

Study Design: Subjects were recruited at three sites. At one site, Caucasian newborns were recruited in a case-control design, while African-Americans were recruited in a cross-sectional design at two additional sites. Genotype at 5 SNPs in the leptin gene was determined for all newborns. Using either birth weight Z score or log(leptin) as dependent variables, ANOVA was performed using a model composed of potential correlates of birth weight or leptin levels (gestational age, gender, size for gestational age, SNP genotype) with an interaction term included to detect a differential association between the dependent variable and genotype, depending on newborn gender.

Results: Leptin levels were about twice as high in females as in males (14.8 vs. 7.0 ng/ml; $p<0.001$), and leptin levels positively correlated with birth weight ($r2=0.33$; $p<0.001$). Relative to homozygotes for the major allele at a SNP upstream of the leptin gene, heterozygous males exhibited an increase in birth weight and cord leptin levels, while females exhibited a decrease in both variables ($F=4.7$, $p=0.01$ for gender x genotype interaction).

Conclusions: Upstream polymorphism in the leptin gene is associated with opposite trends in birth weight and cord leptin levels in male and female newborns.

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PDE4D and _ALOX5AP_ genes are associated with ischemic stroke. *R. Kaushal[1], D. Woo[2], P. Pal[1], H. Xi[1], K. Alwell[2], P. Sekar[1], M. Haverbusch[2], C. Moomaw[2], B. Kissela[2], L. Sauerbeck[2], R. Chakraborty[1], J. Broderick[2], R. Deka[1].* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Dept Neurology, Univ Cincinnati, Cincinnati, OH.

Stroke is the third commonest cause of death and the leading cause of disability in the United States. It is a complex phenotype with both genetic and environmental risk factors interacting in its etiology. Genetic variations in the phosphodiesterase 4D (*PDE4D*) and the 5-lipoxygenase activating protein (*ALOX5AP*) genes have been shown to be associated with stroke and its sub-types in Icelandic and European populations. We report the results of association of six SNPs in *PDE4D* and five SNPs in *ALOX5AP* with various subtypes of stroke in Caucasians. We enrolled cases (N = 252 ischemic subtypes that include cardioembolic, large vessel, small vessel and unknown; 87 subarachnoid and 185 intracerebral hemorrhage), and race and age matched population controls (N = 519) from the greater Cincinnati area. Allelic variant *rs152312* in *PDE4D* is associated with cardioembolic (*P* = 0.006) and small vessel stroke (*P* = 0.026). We inferred haplotypes using the HelixTree software and detected significant association of *PDE4D* haplotypes with cardioembolic and large vessel stroke (*P* 0.0001). No association is observed with the subtypes of hemorrhagic stroke. In *ALOX5AP*, SNP *rs4769874* is significantly associated with ischemic stroke (*P* 0.0001) and subtypes cardioembolic (*P* 0.0001) and large vessel stroke (*P* = 0.0005). This variant was also found significantly associated with ischemic stroke in the Icelandic and a Central European population. We found nominal association of *ALOX5AP* SNP *rs9579646* with ischemic (*P* = 0.022) as well as subarachnoid hemorrhage (*P* = 0.02) stroke. Haplotype analysis showed strong association of *ALOX5AP* with cardioembolic and large vessel stroke (*P* 0.0001); however, we do not observe any association with hemorrhagic stroke. Our study confirms that polymorphisms in *PDE4D* and *ALOX5AP* significantly contribute to the risk of ischemic stroke among the Caucasians. This study was supported by NIH grants NS36695 and ES06096.

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Hemorrhagic stroke and variants in the *ACE* gene. *P. Pal[1], D. Woo[2], R. Kaushal[1], H. Xi[1], M. Haverbusch[2], P. Sekar[1], C. Moomaw[2], L. Sauerbeck[2], R. Chakraborty[1], J. Broderick[2], R. Deka[1].* 1) Department of Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Department of Neurology, Univ Cincinnati, Cincinnati, OH.

Hemorrhagic stroke, which results from rupture of intracranial vessels, is subdivided into intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). ICH has an incidence of 15-19 per 100,000 and SAH has an incidence of 6-8 per 100,000 persons in Western Europe and North America. Both ICH and SAH has a 30-day mortality rate exceeding 40%. Environmental and genetic factors are associated with the occurrence of both ICH and SAH. Association of the ACE gene (*ACE*) with hypertension, a proven major risk factor for both ICH and SAH, has been demonstrated. *ACE* has also been implicated in hemorrhagic stroke. However, most studies have reported only the insertion/deletion (*I/D*) polymorphism and the results of association are inconsistent. We tested for allelic and haplotype associations using six variants in *ACE* with hemorrhagic stroke in a sample of Caucasian cases (N = 185 ICH and 112 SAH) and population-based race and age-matched controls (N = 367 ICH and 217 SAH) from the greater Cincinnati region. The *I/D* polymorphism as well as the other SNPs showed no association with ICH or SAH either at allelic or genotypic level. However, haplotypes inferred using the HelixTree software showed significant association with both SAH ($P = 0.011$) and ICH ($P = 0.008$). When ICH cases were divided into lobar (N = 65) and non-lobar (N = 115) cases, no significant associations were found either at allelic or genotypic level; however, significant haplotype associations were observed for both subtypes (lobar $P = 0.005$; non-lobar $P = 0.019$). Our study suggests that *ACE* plays a plausible role in the pathogenesis of hemorrhagic stroke and warrants a rigorous examination of larger number of variants within the gene. Supported by NIH grants NS36695, ES06096.

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Association study of OPRM1 with smoking initiation and progression to nicotine dependence. *L. Zhang[1,2], K.S. Kendler[1], X. Chen[1].* 1) Virginia Institute for Psychiatric and Behavioral Genetics and Department of Psychiatry , Virginia Commonwealth University, Richmond, VA; 2) Psychiatry Department, West China Hospital, Sichuan University, Chengdu, China.

The gene encoding the mu-opioid receptor (OPRM1) has been reported associated with a range of substance dependences. Experiments in knockout mice implicate that the mu-opioid receptor may mediate reinforcing effects of nicotine. In human, opioid antagonist naltrexone may reduce the relative reinforcing effects of nicotine via cigarette smoking.The OPRM1 is thus a plausible candidate gene influencing smoking behavior. To investigate whether OPRM1 contributes to the susceptibility of smoking initiation and progression to nicotine dependence, we selected 6 haplotype-tagged single nucleotide polymorphisms (SNPs) in this gene and genotyped 688 subjects of lifetime smokers and nonsmokers. We performed chi-square tests for both allelic and genotypic associations and failed to find significant results for neither smoking initiation nor nicotine dependence. Haplotype analyses based on expectation-maximization algorithm showed no significant associations. These preliminary results provide no support for the hypothesis that OPRM1 contributes to susceptibility to smoking initiation and progression to nicotine dependence.

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Promoter polymorphisms in the apolipoprotein H (APOH) gene and the risk of systemic lupus erythematosus. *E. Jacobs[1], Q. Chen[1], M. Kenney[1], P. Shaw[2], A. Kao[2], C. Kammerer[1], F. Bontempo[3], S. Manzi[2], M.I. Kamboh[1].* 1) Department of Human Genetics; 2) Division of Rheumatology & Clinical Immunology; 3) Department of Medicine, University of Pittsburgh, Pittsburgh PA.

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease. Almost 50% of the SLE patients have antiphospholipid autoantibodies (APA) in their sera. APOH is a major autoantigen for the production of APA and thus genetic variation in the APOH gene may affect the risk of SLE. In this study we tested the hypothesis that single nucleotide polymorphisms (SNPs) in the APOH promoter are associated with SLE, the severity of SLE, and the presence of APA. Three hundred and eighty women with SLE (334 Caucasians and 46 African Americans) and 497 healthy control women (455 Caucasians and 42 African Americans) were genotyped for 6 SNPs in the APOH promoter using pyrosequencing. The presence of APA was determined using fluorometric assays. Association studies were carried out using the 2 distribution and Z-tests. The EH program was used for the haplotype analysis. The distribution of four of the 6 SNPs differed significantly between Caucasians and African Americans. Individually, none of the promoter SNPs differed significantly between cases and controls in Caucasians; however the SNPs at positions -700 and -759 had differences in genotype distributions between cases and controls in African Americans (-700, p=0.035; -759, p=0.022). Six-site haplotype analysis was performed among Caucasians only because of the relatively large number of cases and controls in this racial group. A total of 26 different haplotypes were observed and the overall haplotype distribution was significantly different between SLE cases and controls (p=0.00015). Additional analyses indicated that the -700 and -759 SNPs were associated with increased renal disease among Caucasian SLE patients (p=0.024; p=0.028, respectively). No significant associations were found between the promoter polymorphisms and the occurrence of APA.

In conclusion, our data suggest that genetic variation in the APOH promoter may be associated with SLE risk, especially risk of renal disease.

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Association of the ubiquilin 1 (*UBQLN1*) SNPs with late-onset Alzheimers disease in a large case-control sample.

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In addition to the *APOE* gene on chromosome 19, several chromosomal regions have been implicated to harbor putative genes for late-onset Alzheimers disease (LOAD), but their identity is not known with certainty. The gene coding for ubiquilin 1 (*UBQLN1*) is located near a linkage peak on chromosome 9q22.2, and it also impacts the function of the presenilin 1 and 2 genes that cause early-onset AD. Thus, *UBQLN1* is both a positional and biological candidate gene for AD. Recently genetic variation in the *UBQLN1* gene has been shown to affect the risk of AD in two independent family-based samples (NEJM 2005; 352:884-894). The purpose of this study was to confirm the reported association in a large case-control sample. We examined the associations of three SNPs in the *UBQLN1* gene (rs2780995, rs12344615 and rs2781002) in 785 LOAD cases and 726 controls. All SNPs were in significant linkage disequilibrium ($p < 0.001$). Significant associations were observed with the rs12344615 and rs2781002 SNPs. The age, sex and *APOE* adjusted odds ratios between the less common allele carriers and wild type homozygous were 1.38 (95%CI: 1.06-1.80; $p = 0.016$) for the rs12344615 SNP and 1.28 (95%CI: 1.00-1.64; $p = 0.048$) for the rs2781002 SNP. These odds ratios are within the range recently reported in the family-based study. Three-site haplotype analysis identified one haplotype to be significantly more common in cases than controls (20.1% vs. 13.8%; $p = 0.0002$) and this haplotype was mainly defined by the risk allele of the rs12344615 SNP.

In summary, our data from a large case-control cohort indicate that genetic variation in the *UBQLN1* gene has a modest effect on AD risk and these results are similar to those found in a recent family-based association study.

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Investigation of the relationship of candidate genes on chromosome 10 with the risk and age-at-onset of Alzheimers disease. *A. Ozturk[1], S.T. DeKosky[2], M.I. Kamboh[1].* 1) Department of Human Genetics; 2) Department of Neurology, University of Pittsburgh, Pittsburgh PA.

Late-onset Alzheimers disease (LOAD) is a complex and multifactorial disease with a substantial genetic component. The APOE*4 allele in the APOE gene is the only established risk factor in the development of LOAD that accounts for 20-30% of the disease risk. Recently, linkage studies in multiplex families with LOAD have provided evidence for the existence of additional putative genes for LOAD on several chromosomes, including chromosome 10. A broad linkage peak encompassing 50 cM region between chromosome 10q21 and 10q25 has been implicated that influence both disease risk and age-at-onset (AAO). There are a number of promising candidate genes in this region that are involved in either the production, processing or clearance of A peptide and include CHAT, PLAU, IDE, HHEX, GSTO1, GSTO2, and PRSS11. In this study, we have examined the role of these genes under the linkage peak on chromosome 10 to assess their role with AD risk and AAO. Association studies for 14 SNPs were performed in a large case-control cohort comprising 1012 white LOAD subjects and 771 white control subjects. No significant associations were observed with any of the SNPs examined in the IDE, HHEX, GSTO1, GSTO2 and PRSS11 genes. Of the 3 CHAT SNPs examined, we detected significant association of the intron 9 SNP with AD risk in both the total sample OR = 2.37, 95% CI: 1.19-4.73; p=0.01) and among non-APOE*4 carriers (OR=2.94, 95% CI: 1.33-6.51; p=0.007). Three-site haplotype analysis confirmed that haplotypes determined by the CHAT/intron 9 SNP were associated with either risk (p=0.0009) or protection (p=0.0082) among non-APOE*4 carriers. In the PLAU gene, we examined 3 tagSNPs and found a modest protective effect with one SNP in the 3UTR (OR=0.71, 95% CI: 0.53-0.95; p=0.02). Suggestive associations for AAO were observed with the PLAU/3UTR (p=0.036) and PLAU/intron 9 (p=0.01) SNPs. In summary, our data on a large number of AD cases and controls suggest that genetic variation in two positional candidate genes on chromosome 10 (PLAU and CHAT) or nearby genes may affect the risk and AAO of LOAD.

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Association of paraoxonase-1 (PON1) polymorphisms with lupus nephritis. *L. Tripi[1], Q. Chen[1], M. Kenney[1], P. Shaw[2], A. Kao[2], C. Kammerer[1], F. Bontempo[3], S. Manzi[2], M.I. Kamboh[1].* 1) Department of Human Genetics; 2) Division of Rheumatology & Clinical Immunology; 3) Department of Medicine, University of Pittsburgh, Pittsburgh PA.

Systemic lupus erythematosus (SLE) is the prototypic systemic inflammatory autoimmune disease with a significant genetic component. About 50% of the SLE patients are positive for antiphospholipid antibodies (APA) and the risk of coronary artery disease (CAD) is up to 50 times higher than the general population. Several lines of evidence suggest the existence of a relationship between oxidative parameters of LDL and APA in SLE patients. PON1 inhibits LDL oxidation and thus may affect the risk of SLE by modifying susceptibility to CAD. In this study we examined the role of 9 SNPs in the PON1 gene in relation to SLE risk. DNA samples from 380 women with SLE (334 Caucasians and 46 African Americans) and 497 healthy control women (455 Caucasians and 42 African Americans) were studied. Association studies were performed using logistic regression. Haplotype analysis was performed using EH program. The genotype distributions of 7 SNPs were significantly different between Caucasians and African Americans. All SNPs were in significant linkage disequilibrium (LD) in the Caucasian sample and all except 2 were in significant LD in the African American sample. The SNP at position -1739 had a significantly different genotype distribution between lupus patients and controls in the Caucasian sample (OR) =0.66, 95% CI 0.45-0.97; p=0.03). The overall haplotype distribution in the Caucasian sample also differed between SLE cases and controls (p=0.051). Stratification of the lupus sample based on renal disease (n=81) and no renal disease (n=243) revealed significant associations with three additional promoter SNPs with ORs 3.82 (95% CI: 1.49-9.82; p=0.005), 3.41 (95% CI: 1.35-8.61; p=0.009) and 2.17 (95% CI: 1.01-4.65; p=0.049). Because these three SNPs were in high LD (|D| range 0.915-0.962), the significant effect is most probably derived from one functional SNP. In conclusion, our data suggest that genetic variation in the PON1 gene may affect the risk of developing lupus nephritis, especially among Caucasians.

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Association of NOD1 Polymorphisms with Atopic Eczema and Related Phenotypes. *T. Illig[1], N. Klopp[1], L. Rummler[2], S. Wagenpfeil[3], N. Novak[4], HJ. Baurecht[3], W. Groer[2], U. Darsow[2], J. Heinrich[1], T. Schafer[5], T. Jakob[2,6], H. Behrendt[6], HE. Wichmann[1], J. Ring[2,6], S. Weidinger[2,6].* 1) Institute of Epidemiology, GSF National Research Center for Environment and Health, Munich, Germany; 2) Department of Dermatology and Allergy Biederstein, Technical University Munich, Germany; 3) Institute for Medical Statistics and Epidemiology, Technical University Munich, Germany; 4) Department of Dermatology and Allergy, University of Bonn, Germany; 5) Institute of Social Medicine, Medical University, Luebeck, Germany; 6) Division of Environmental Dermatology and Allergy GSF/TUM, GSF National Research Center for Environment and Health & ZAUM Center for Allergy and Environment, Technical University Munich, Germany.

Interactions with microbial pathogens are crucial for the maturation of the immune system. The nucleotide-binding oligomerization domain protein 1 (NOD1) is a cytosolic receptor sensing a muropeptide found mostly in gram-negative bacterial peptidoglycans. NOD1 is located on chromosome 7p14-p15, a region which has been linked with atopy. Within a large population-based cohort of German adults (n=1417), a case-control population for atopic eczema (n=454), and a large cohort of parent-offspring trios for atopic eczema (189 trios), we evaluated 11 NOD1 polymorphisms for associations with atopic phenotypes. Analyses revealed significant association of one NOD1 haplotype with atopic eczema in the population-based cohort (p=0.004) and the case-control population (p=0.003). Another NOD1 haplotype (p=0.008) was associated with increased total IgE. In addition, significant associations with total serum IgE levels were observed for polymorphisms rs2907748 (p=0.006), rs2907749 (p=0.012) and rs2075822 (p=0.018). These polymorphisms were significantly associated with atopic eczema and asthma in the family-based association analyses (p=0.001-0.043). Seven polymorphisms showed significant transmission distortion for total IgE levels (p-values < 0.0001 - 0.029). These data indicate that genetic variants within NOD1 are important determinants of atopy susceptibility.

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Using HapMap methods and data in lung cancer study. *Y. Gao[1], T. Sun[2], S. Ma[1], W. Tan[2], Q. Zhang[1], D. Lin[2], C. Zeng[1].* 1) Beijing Genomics Institute, The Chinese Academy of Sciences, Beijing Airport Industrial Zone B6, Beijing, 101300, China; 2) Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 17 Panjiayuan Nanli, Beijing, 100021, China.

In an attempt to mine genes and their polymorphisms correlated to lung cancer, 4 chromosomal regions were chosen for SNP genotyping and association studies in case and control samples from 1800 individuals. Initially, 97 SNPs were selected and genotyped in 192 case and 192 control samples. Then 58 SNPs with MAF>0.05 were chosen to genotype in enlarged samples from total of 923 case and 888 control individuals. In a 26 kb region on chromosome 10, 14 SNPs were genotyped whereas using various types of analysis neither SNP nor haplotype was shown to associate with the cancer. 22 SNPs were genotyped in chromosomal region 2 which spans 57 kb. Three SNPs appeared to be associated with moderately increased risk of the disease by comparing individual SNPs and haplotype patterns of case and control samples. However, after partitioning the haplotype blocks, a haplotype containing 13 typed SNPs, with a frequency of 19.5%, appears strongly associated with lung cancer. In a 9-kb region which contains only one gene, 8 novel SNPs were discovered by resequencing. Three SNPs and one haplotype were detected to be associated with cancer. Region 4 on chromosome 2 covers 200 kb. 14 SNPs which are defined as tag SNPs in HapMap data were selected for genotyping. 5 of 14 SNPs appear to correlate with the cancer. Moreover, 2 blocks, containing 18 haplotypes, were identified in all samples. One haplotype appears to correlate to lung cancer significantly.

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Replicated linkage and association between *DRD3* and schizophrenia: Attempting to resolve a history of inconsistencies. *M. Talkowski[1], H. Mansour[1], J. Wood[1], S. Deshpande[2], B.K. Thelma[2], K.V. Chowdari[1], V.L. Nimgaonkar[1].* 1) Univ of Pittsburgh, Pgh, PA; 2) Dr. RML Hospital and Univ of Delhi, New Delhi, India.

Over forty studies have evaluated a single coding SNP, rs6280, at *DRD3* for associations with schizophrenia (SZ). Yet, the results remain inconclusive. Simulations have suggested variants in LD with an unidentified liability locus may produce inconsistent associations. Thus, we investigated additional SNPs within and flanking *DRD3*. Our results suggest replicated associations in two samples, and post-hoc LD analyses indicate the associations are independent of rs6280.

We initially analyzed 13 SNPs spanning 109kb in 331 SZ cases, including 151 case-parent trios, and 274 controls from the US. Subsequently, we evaluated 11 SNPs in an Indian cohort (141 trios). In the U.S. sample, we detected associations with eight SNPs, including rs6280 ($p=0.001$). Notably, five contiguous SNPs spanning intron 1 to the 3 region were associated with SZ. Associations were observed with haplotypes incorporating these SNPs, particularly a common haplotype in both case-control ($p=0.002$) and family-based designs ($p=0.00009$). In the Indian cohort, we detected associations with one SNP (rs10934254; $p<0.05$), and the identical haplotype spanning the gene ($p=0.009$). Global tests of transmission distortion supported associations in both samples ($p=0.009$ and 0.005).

To evaluate if our results were attributable to LD with rs6280, or other variants, we sought comprehensive LD analyses. Direct sequencing of 64.7kb (including 8.5kb 5, 12.9kb 3) in a pool of 400 people to identify polymorphisms is ongoing. At the time of submission, we identified 62 SNPs, including 13 novel SNPs. Sequencing of 30 CEPH individuals reveals rs6280 clusters with 4 SNPs ($r^2>0.8$), all 5 to exon 1, and was not correlated any common SNPs 3' to exon 1. We find that 18 tags would represent all common SNPs detected thus far. Our results indicate novel SNPs at *DRD3* may contribute to SZ pathogenesis. Post-hoc LD analyses suggest comprehensive evaluation of this gene has not yet been achieved in association studies. Such analyses are ongoing in a large cohort and may further characterize these results.

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Insulin Resistance and Inflammation: Cause or Effects? *X. Guo[1], K.D. Taylor[1], M.J. Quinones[2], J. Cui[1], I. Bulnes-Enriquez[2], R.D.L Rosa[2], H. Yang[1], W. Hsueh[2], J.I. Rotter[1].* 1) Medical Genetics Inst, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Dept of Medicine, UCLA, Los Angeles, CA.

Recent data have shown that plasma inflammatory markers predict the development of diabetes and have supported the hypothesis that low-grade inflammation is part of the insulin resistance (IR) syndrome. The relevant question is whether the inflammatory process precedes IR. Support for this possible sequence would come from the demonstration of the association of variation in inflammatory genes with IR. Thus, the aim of this study was to investigate the role of genetic variants in the C-reactive protein (CRP) gene in IR. Families were ascertained via a coronary artery disease proband in the Mexican-American Coronary Artery Disease Project. Individuals from 167 Mexican-American families (n=894) were genotyped for 6 polymorphisms in the CRP gene using the Taqman MGB assay. Adult offspring and offspring spouses (423 individuals from 100 families) were phenotyped for insulin sensitivity by the gold standard for insulin resistance, the hyperinsulinemic euglycemic clamp. One SNP was not polymorphic in this population, so the analyses were performed on the remaining 5 SNPs. Association was tested using the general estimating equations methods. After adjusting for age, sex, and body mass index, SNP rs3093062 was found to be associated with both insulin sensitivity index (SI, p = 0.008) and glucose infusion rate (GINF, p = 0.003); while rs3093066 and rs2808630 were associated with GINF (p = 0.04 and 0.001, respectively). Haplotype analysis based on the 5 SNPs revealed that all 5 SNPs were in the same haplotypic block, and that the second common haplotype, which is distinguished by the rare variant in SNP rs2808630, was strongly associated with IR as indicated by a lower mean GINF (mean ¡À SE: 370 ¡À 13 for H2 carriers vs. 417 ¡À 12 for non-carriers, p = 0.0005). These results suggest that genetic variants in the CRP gene may contribute to IR and support the hypothesis that inflammatory genes may link chronic inflammation and insulin resistance.

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Association of functional polymorphisms in neuronal tryptophan hydroxylase gene with bipolar affective disorder. *H.S. Sun[1], Y.M.J. Lin[2], S.C. Chao[1], T.J. Lai[3], T.M. Chen[2].* 1) Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan; 2) The Institute of Basic Medical Sciences, National Cheng Kung University Medical College, Tainan, Taiwan; 3) Department of Psychiatry, Chung Shan Medical University Hospital, Taichung, Taiwan.

Disturbances in serotonin neurotransmission have been implicated in the etiology of many psychiatric disorders including bipolar affective disorder (BPD). The tryptophan hydroxylase 2 gene (*TPH2*) encodes the rate-limiting enzyme in neuronal serotonin biosynthetic pathway, thus it is one of the leading candidate genes for psychiatric and behavioral disorders. This study was designed to investigate the role of *TPH2* gene in the pathophysiology of BPD. We have systematically screened the sequence of human *TPH2* gene and identified 10 sequence variants in Taiwanese Han population. Five SNPs that either with potentially functional relevance or moderate minor allele frequencies were selected for association study between 106 BPD cases and 105 matched control samples. Significant differences in single-locus allele distributions and haplotype profile of *TPH2* polymorphisms between case and control groups were found. The involvements of common variants on *TPH2* gene in BPD etiology were evidently by functional assays of two SNPs on promoter and coding region that affect the TPH2 expression level and the enzyme activity, respectively. Results of this study indicate the *TPH2* gene has biological and functional significance in BPD etiology, and provide the knowledge of common variants effect on the physiological status in vivo.

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Genetic variations and disease-related genes in the Korean population studied by scanning the cytokine cluster region of chromosome 5q31-33. *B. Oh[1], J-K. Lee[1], H-J. Ryu[1], H-T. Kim[1], J-Y. Lee[1], H.D. Shin[2], C-S. Park[3], K. Kimm[1].* 1) Structural & Funct Genomincs, Natl Genome Research Inst, Seoul; 2) SNP Genetics, Inc., Seoul; 3) Genome Research Center for Allergy and Respiratory Diseases, Soonchunhyang University, Bucheon.

Numerous genetic studies have mapped disease genes to a region on chromosome 5q31-33 containing a cluster of cytokines. In order to determine the genetic variations and the haplotypes of genes in the chromosome 5q31-33 region, we resequenced all exons and the promoter regions of 130 genes in 24 unrelated Koreans. We identified a total of 1,829 genetic variants, including 1,560 single nucleotide polymorphisms (SNPs), 106 insertion/deletion polymorphisms and 163 microsatellites. Among these results the analysis of 289 SNPs derived from 29 genes was carried out. The standardized variance (FST) for allele frequency showed substantial differences among different ethnic groups. Using FST as a genetic distance, remarkable differences were observed between Korean and Caucasian or African (median FST=0.036; 0.038, respectively), whereas high similarity was found between Japanese and Korean (median FST = 0.002). Using these SNP information association study has been pursued to identify genes responsible for asthma. TaqMan and Illumina platform have been used for genotyping case and matched control DNAs. Among genes analyzed initially DCNP1 showed significant association with specific serum IgE levels in asthmatic patients. The minor allele of -1288C>T showed strong protective effects on the specific IgE production among asthmatic patients (P=0.0009, OR=0.63). On the other hand, two SNPs in 3'UTR (+1718C>T and +2666C>A) revealed the susceptibility on the IgE production (P=0.31-0.003, OR=1.37-1.82). 130 genes in 5q31-33 have been under the statistical analysis for their association to asthma and its subphenotypes such as IgE levels. Our results will provide the valuable evidence for gene-based analysis for association studies as well as the useful information for the identification of immune-related disease genes in the chromosome 5q31-33.

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Epistatic effects in hyperserotonemia and autism. *A.M. Coutinho[1], G. Oliveira[2], I. Sousa[1], T. Morgadinho[3], C. Fesel[1], T.R. Macedo[3], C. Bento[2], M. Martins[1,5], C. Marques[2], A. Ataíde[4], T.S. Miguel[4], L. Borges[2], A.M. Vicente[1,5].* 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Portugal; 3) Faculdade de Medicina da Universidade de Coimbra, Portugal; 4) Direcção Regional de Educação da Região Centro, Coimbra, Portugal; 5) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal.

One of the most studied subphenotypes in autism is platelet hyperserotonemia, which is likely influenced by several genes in a non-additive manner. A genome scan for QTLs determining serotonin (5-HT) levels found a linkage peak at 17q11-21, where the 5-HT transporter gene (*SLC6A4*) and the integrin 3 chain precursor gene (*ITGB3*) map, and a previous analysis of our autism sample showed an association of specific *SLC6A4* variants with hyperserotonemia. In this study, we further assess the genetic basis of hyperserotonemia in 103 autistic children. Epistasis among six candidate genes (*SLC6A4*, *HTR2A*, *HTR1D*, *HTR1A*, *TPH1* and *ITGB3*) was tested using the Restricted Partition Method (RPM). We found significant interactions between haplotypes in *HTR1A* and *ITGB3* (P=0.004) and between haplotypes in *SLC6A4* and *HTR2A* (P=0.005). While haplotypes in *SLC6A4* and *ITGB3* were independently associated with 5-HT levels, with specific variants increasing risk for hyperserotonemia, interaction between *SLC6A4* and *ITGB3* was more modest (P=0.015). This suggests that these genes may have an independent main effect and interact with others in the regulation of 5-HT levels. A strong linkage peak in this same 17q11-21 region has been reported for autism, suggesting that the occurrence of hyperserotonemia in autism may be mediated by genetic variation at the *SLC6A4* and *ITGB3* genes. We therefore used the Multifactor-Dimensionality Reduction (MDR) program to test for epistasis in autism among the markers shown to interact for the determination of 5-HT levels. In a sample of 167 autistic patients and 144 controls, we found a significant three-locus interaction among polymorphisms in *ITGB3* and *SLC6A4* (P=0.021). Our results indicate that the interaction of gene variants involved in hyperserotonemia is also significantly associated with increased risk for autism.

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APOE in multiple sclerosis (MS) susceptibility and severity: meta- and pooled analyses. *R. Burwick[1], P.P. Ramsay[1], J.L. Haines[1], M.A. Pericak-Vance[1], S.L. Hauser[1], S. Schmidt[1], S. Sawcer[2], A. Compston[2], G. Savettieri[3], A. Quattrone[3], J. Zwemmer[4], S. Weatherby[5], F. Fazekas[6], R. Schmidt[6], C. Enzinger[6], J. Hillert[7], T. Masterman[7], P. Hogh[8], P. Maciel[9], L.F. Barcellos[1].* 1) US MS Genetics Group, UCSF; 2) Univ of Cambridge, UK; 3) Univ of Palermo and Magna Graecia, IT; 4) Univ Med Center, NL; 5) Derriford Hosp, UK; 6) Med Univ Graz, AT; 7) Karolinska Inst, SE; 8) Copenhagen Univ Hosp, DK; 9) Univ of Minho, PT.

Variation in MHC genes on chr.6p21, specifically the HLA-DRB1*15 haplotype, has been convincingly shown to confer MS risk. Whole genome screens have previously suggested chr.19q13 as a potential region harboring additional MS genes. APOE, a candidate gene located here, encodes a major lipid carrier protein in the brain and has long been associated with regeneration of axons and myelin. Previous studies have examined APOE variation in MS, but have shown conflicting results and have lacked statistical power to detect modest genetic influences. Because of the importance of identifying risk and prognostic factors in MS, we performed meta- and pooled analyses using large sample sizes to determine whether APOE variation influences risk, disease severity or other clinical outcomes. Using 2,900 (each) cases and controls, we observed no effect of e2 or e4 allele carrier status on MS risk (OR=1.10; 95% CI=0.94-1.30; p=0.24 and OR=0.89; 95% CI=0.78-1.02; p=0.09, respectively), nor were differences in genotypic distributions detected. Furthermore, results obtained from family-based analyses of APOE (1281 MS families/1152 trios/721 DSP) were also negative (PDT, p=0.62). Finally, results obtained from pooled analyses of 3,500 MS cases also argue strongly that APOE variation does not (1) distinguish a relapsing-remitting from primary progressive course, (2) influence severity, as measured by EDSS and duration, or (3) influence age of onset or gender distribution. While this study cannot exclude an APOE effect on other measures of progression that were not examined here, including subclinical MRI phenotypes; these findings do not support a role for APOE in MS, and highlight the need for adequate sample sizes, particularly in studies of genotype-phenotype relationships.

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Association analysis of interleukin 4 receptor and interleukin 13 polymorphisms indicate a difference in development of atopy expression leading to atopic asthma or allergic rhinitis. *R.W.B. Bottema[1,2], I.M. Nolte[1], T.D. Howard[3], G.H. Koppelman[2], D.A. Meyers[3], A.E.J. Dubois[2], E.R. Bleecker[3], D.S. Postma[1,2].* 1) University of Groningen, Netherlands; 2) University Medical Center Groningen, Netherlands; 3) Wake Forest University School of Medicine, Winston-Salem.

Background: It is unknown why atopy is associated with asthma in one individual and with rhinitis in another. Genetic polymorphisms may explain this. We and others previously showed that single nucleotide polymorphisms (SNPs) of the interleukin 4 receptor (IL4R) and interleukin 13 (IL13) were associated with asthma and atopy traits. **Objective**: To study the IL4R and IL13 SNPs in relation to allergic rhinitis, asthma and atopy traits in rhinitis and asthma patients. **Methods**: 4 IL4R and 3 IL13 SNPs were studied in three independent Dutch populations: 1) 226 nuclear families ascertained through a proband with allergic rhinitis; 2) 407 nuclear families with an asthma proband; 3) 120 asthmatic individuals and 105 unrelated healthy controls. Family based association testing with atopy, asthma and rhinitis phenotypes was performed. Case-control analyses using 2 tests and Kruskall-Wallis tests were performed as appropriate. **Results**: IL13 SNP G870A was consistently associated with asthma, airway hyperresponsiveness (AHR) and serum IgE in both asthmatic populations ($p<0.05$). IL4R SNP E375A and S411L were associated with asthma and AHR in the asthma case-control population ($p<0.05$). All SNPs were not significantly associated with allergic rhinitis. Remarkably, within the rhinitis population there was no association with the traits AHR and serum IgE. **Conclusion**: Genetic polymorphisms of IL4R and IL13 are associated with asthma, AHR and atopy in an asthmatic population, but not in a rhinitis population. The results may indicate that atopy in asthmatics is genetically differently regulated from atopy in rhinitis patients. Further study is warranted, with high power to prove the absence of association in rhinitis patients.

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The DRD2 C957T polymorphism and its interaction with COMT Val158Met polymorphisms in human working memory ability. *H. Xu[1], C.B. Kellendonk[2], E.H. Simpson[2], J.G. Keilp[3], G.E. Bruder[3], H.J. Polan[2], E.R. Kandel[2], T.C. Gilliam[1, 3].* 1) Department of Genetics and Development, Columbia University, New York, NY; 2) Center for Neurobiology and Behavior, Columbia University, New York, NY; 3) Department of Psychiatry, Columbia University, New York, NY.

Working memory impairment is a core cognitive deficit in schizophrenia, and dopamine neurotransmission plays a critical role in both normal cognitive processing and schizophrenia. In a sample of 402 healthy adults assessed on 4 working memory tasks, we observed the association of a polymorphism (Val158Met) of COMT, an enzyme essential for dopamine metabolism in the prefrontal cortex, with the performance on the WAIS-III letter number sequencing test (Bruder et al., in press). In a follow up study we genotyped a polymorphism in another dopaminergic gene, the human dopamine receptor D2 (DRD2) gene. The C allele of a synonymous SNP (C957T) in DRD2 has been shown to increase striatal DRD2 binding potential in vivo (Hirvonen et al., 2004) and to be associated with schizophrenia (Lawford et al., 2005). Our mouse studies indicate that over-expression of DRD2 in the striatum leads to impairments in working memory tasks (Kellendonk et al., submitted). We genotyped C957T in the Caucasian subset of our sample to assess its relation to human working memory ability. A significant impairment in the word serial position test (WSPT) performance was found in subjects with the C/C genotype versus the other two genotypes (ANOVA p=0.04). Among the 6 serial positions (varying in difficulty level) in the WSPT, only accuracy at the 5th position differed significantly between these two groups (ANOVA p=0.005). When interaction with the COMT Val158Met polymorphism is included in the analysis model, the relation of the C957T genotype to WSPT Performance (ANOVA p=0.01) and accuracy at the 5th position (ANOVA p=0.001) was strengthened. Our data suggest that the DRD2 C957T polymorphism affects human working memory. Further, they suggest that it is important to analyze genetic interactions between different polymorphisms for the understanding of complex phenotypes such as cognitive function.

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Estrogen receptor polymorphisms and premature ovarian failure. *K. Bretherick[1], L. Currie[1], M. Fluker[2,3], W. Robinson[1].* 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Obstetrics and Gynaecology, Univ British Columbia, Vancouver, BC, Canada; 3) Genesis Fertility Center, Vancouver, BC, Canada.

Although the average age of menopause is 51 years, approximately 1% of women will experience menopause before the age of 40, a condition known as premature ovarian failure (POF). POF is the cessation of ovulation and menstruation, caused by an absence of folliculogenesis in the ovary. Estrogen acts through estrogen receptor- (ER-) at the hypothalamus to stimulate the release of cyclic gonadotropins that regulate follicular proliferation, and through estrogen receptor- (ER-) in the ovary to directly enhance follicular differentiation. Genetic variants affecting expression or function of the ER- and ER- genes may therefore be associated with POF. We examined allele frequencies of a TA repeat in the promoter of the ER- gene and an intronic CA repeat in the ER- gene in women with idiopathic POF and control women using PCR amplification and fragment analysis on an ABI310 genetic analyzer. A clear bimodal distribution of ER- alleles is observed in both patient and control groups with short alleles (18 repeats) being significantly less common in the POF patient population (43%, N=108) than among the control women (60%, N=218) (p=0.004, ^{2}). Perhaps most striking is that genotypes consisting of two short alleles were found in only 3 of 54 (6%) POF patients and in 40 of 109 (37%) control women (p0.0001, ^{2}). This suggests that long repeats at the ER- gene may predispose to POF in a dominant fashion. The ER- TA repeat may have a direct effect on expression of the ER- gene or alternatively it may be in linkage disequilibrium with a functional variant in the ER- gene. The allele distribution of ER- CA repeats was not significantly different in the POF patient and control populations. These results support an association between POF and the ER- TA repeat but not the ER- CA repeat polymorphism, suggesting that the stimulatory affect of estrogen on the hypothalamus is critical in maintaining folliculogenesis.

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Identifying the genetic factors involved in radiation-induced pulmonary fibrosis. *M. Begin, T. O'Brien, G. Dorion, C. Haston.* Dept Human Genetics, McGill Univ, Montreal, PQ, Canada.

Radiation treatment to the thoracic cavity results in a severe inflammatory and/or fibrotic response in 5% of exposed individuals. Identifying the susceptibility genes using a mouse model will allow for the identification of susceptibility loci in humans and will permit pre-radiation screening. Previous studies have shown that there are three potential susceptibility loci involved in the fibrosis response in C57Bl/6J x C3Hf/Kam F_2 mice; *Radiation-Induced Pulmonary Fibrosis 1* (*Radpf*) on chromosome 17, *Radpf2* on chromosome 1, and *Radpf3* on chromosome 6. In this study, the radiation responses of a cohort of 149 mice, bred to contain 6.25% C3H alleles, including the regions of *Radpf1-3* in a 93.75% C57Bl/6J background were measured. Mice were treated with 18 Gy of Cesium radiation to the whole thoracic cavity and sacrificed at 6 months post irradiation treatment or when moribund. Histological analysis of the left lung provided the percentage of the lung that contained the pulmonary fibrosis phenotype. Mice became sick between 11-26 weeks post irradiation and the average sacrifice time for the mice was 22 weeks. The mice had a range of 0%-30% pulmonary fibrosis on their lungs, with an average diseased area of 2.56%. Mice were genotyped using 25 markers on chromosomes 1,6, and 17 (7-10 markers each). At *Radpf1* the average pulmonary fibrosis area of mice with B6 alleles was 2.79%0.48, which was significantly greater then the average phenotype of mice with C3H alleles, 0.19%0.16, (P=0.024). Similarly, at *Radpf2* and *Radpf3* mice with B6 alleles had greater fibrosis than mice with C3H alleles (2.81%0.45 versus 0.47%.05, P=0.05) and (2.84%0.05 versus 0.73%0.32, P=0.01), respectively. The linkage on chromosome 6 was significant, after accounting for the linkage on chromosome 17 (P=0.0074). We have provided supporting evidence for the existence of 3 loci which influence the development of radiation-induced pulmonary fibrosis in mice.

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Bleomycin-induced pulmonary fibrosis susceptibility genes in AcB/BcA recombinant congenic mice. *A. Lemay, C.K Haston.* Dept Human Genetics, McGill Meakins-Christie, Montreal, PQ, Canada.

The genetic basis of susceptibility to pulmonary fibrosis is largely unknown. Initially, in this study, loci regulating the response of bleomycin-induced pulmonary fibrosis were mapped using a set of recombinant congenic strains bred from pulmonary fibrosis-resistant A/J and susceptible C57BL/6J (B6) mice. Linkage was identified on chromosomes 9 and 6 (LOD= 4.9 and 2.4) and other suggestive loci were detected. The putative loci include alleles from both the B6 and A/J strains as increasing the fibrosis response of the congenic mice. To further investigate these putative linkages, chromosome substitution strains [C57BL/6J-Chr 6A/NaJ (n=9) and C57BL/6J-Chr 17A/NaJ (n=8)], were treated with the bleomycin protocol and were found to be resistant to bleomycin-induced pulmonary fibrosis (% fibrosis=0.35 0.36 and 0.02 0.06), supporting the existence of A/J-derived protective alleles on these chromosomes. Backcrosses of the recombinant congenic strain BcA84 (resistant to bleomycin-induced pulmonary fibrosis, n=28) were treated with bleomycin and linkage analysis revealed a potential locus on chromosome 6 (LOD=2.3) which was located in the region identified using the recombinant congenic mice. To identify potential fibrosis candidate genes in the putative linkage intervals, gene expression studies with microarrays were completed. This analysis revealed 3304 genes or expressed sequence tags to be differentially expressed ($p < 0.01$) in lung tissue between bleomycin treated B6 and A/J mice, and 246 of these genes to map to the potential susceptibility loci. Pulmonary genes differentially expressed between bleomycin treated B6 and A/J mice included those of heparin binding and extracellular matrix deposition pathways. A review of available genomic sequences revealed 809 (43% of total) genes in the linkage intervals to have variations predicted to alter the encoded proteins or their regulation, 68 (8.4%) of which were also differentially expressed. Genomic approaches were combined to produce a set of candidate genes which may influence susceptibility to bleomycin-induced pulmonary fibrosis in the A/J:B6 mouse model.

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A common mutation in an organic anion transporter gene SLC22A12 is a suppressing factor for the development of gout. *N. Kamatani[1,2], A. Taniguchi[2], W. Urano[2], M. Yamanaka[2], S. Furihata[1,3], M. Hosoyamada[4], H. Endou[5], H. Yamanaka[2].* 1) Div Genomic Med, Tokyo Women's Med Univ, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women'fs Medical University; 3) Genome Diversity Team, Japan Biological Information Research Center, Japan Biological Informatics Consortium; 4) Department of Pharmacotherapeutics, Kyoritsu University of Pharmacy; 5) Department of Pharmacology and Toxicology, Kyorin University School of Medicine.

Purpose: Previous studies have been suggested that various genetic factors are likely to determine the susceptibility of subjects to primary gout. However, studies of the genetic factors of primary gout have seldom been undertaken. Recently, urate transporter 1 (URAT1) encoded by SLC22A12, has been identified as a urate-anion exchanger in humans. It has been demonstrated that URAT1 plays a central role in reabsorption of urate from glomerular filtrate and may be the major mechanism for regulating blood urate levels. In this study, we examined SLC22A12 gene in patients with gout. The purpose was to analyze the roles of SLC22A12 in the development of gout. Method: 185 Japanese male patients with primary gout were randomly selected. The control group was consisted of 594 males and 386 females. The study was focused on G774A mutation in SLC22A12. This mutation leads to the substitution of a stop codon for tryptophan (W258X), and homozygous G774A mutation is the predominant cause of idiopathic renal hypouricemia in Japanese. Results: 25 males and 20 females in the control group were heterozygous for G774A. However, none of gout patients had the mutant allele. A homozygous mutation of G774A was found in neither of the groups. The heterozygotes were observed more frequently in male controls than in the gout patients (P = 0.001). The serum levels of uric acid were significantly lower in subjects with GA genotype than those with GG genotype both in male controls (3.9 '} 0.8 for G/A and 5.8 '} 1.1 for G/G, P0.0001) and female controls (2.9 '} 0.6 for G/A and 4.0 '} 0.8 for G/G, P0.0001). Conclusion: G774A mutation in SLC22A12 gene serves as a suppressing factor for the development of gout.

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Smoking Influences the Association of LEPR SNPs with Body Composition and Bone Mineral Density. *U.L Fairbrother[1], A.J. Walley[1], L.B. Tanko[2], C. Christiansen[2], P. Froguel[1], A.I.F. Blakemore[1].* 1) Section of Genomic Medicine, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, United Kingdom; 2) Centre for Clinical and Basic Research, Ballerup Byvej 222, DK-2750 Ballerup, Denmark.

Leptin is secreted by adipocytes and a range of other cell types, including osteoblasts. Like other cytokines, it is pleiotropic in its effects: reflecting and regulating adiposity; promoting inflammation and modulating bone turnover. Nonsense mutations in the leptin receptor gene (LEPR) cause monogenic obesity and non-synonymous LEPR SNPS have been associated with body composition in a range of studies. One SNP, Q223R, has also recently been associated with peak bone mass in young Chinese women and in young Korean men. We have analysed three SNPs in the extracellular region of the LEPR: K109R, Q223R and K656N, for associations with body composition and bone mineral density (BMD) in a large cohort of postmenopausal Danish women. There were no significant departures from HWE, for any SNP. Associations of Q223R were seen with total hip BMD and with central lean mass (p=0.03 and 0.046). The associations were strongest in smokers: Q223R was associated with BMD in total hip, femoral neck and spine (p=0.0009, 0.001 and 0.007). In addition, Q223R was associated with lean mass (p=0.046), particularly in smokers: peripheral and total lean mass (p=0.02 and 0.015). K109R was associated with hip BMD only in smokers (p=0.021). Overall, results with K656N were consistent with those of Q223R in that this SNP was associated with peripheral and total lean mass (p=0.029 and 0.01). Additionally in smokers, K656N was associated with peripheral and total lean/fat ratios and % fat (p=0.017, 0.008, 0.008). This study indicates that LEPR variation affects lean mass as well as adiposity and also influences bone mineral density in a European population and that this association is more pronounced in smokers. There have been previous reports of interactions of smoking status with LEPR SNPs in the regulation of adiposity, but this is the first report of a similar effect on BMD.

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A spectrum of *PCSK9* alleles contributes to variation in plasma levels of LDL-cholesterol. *I.K. Kotowski, A. Pertsemlidis, J. Cohen, H.H. Hobbs.* UT Southwestern Medical Center, Dallas, TX.

Selected missense mutations in the proprotein convertase subtilisin/kexin type 9 serine protease gene *PCSK9* cause autosomal dominant hypercholesterolemia, whereas nonsense mutations in *PCSK9* are associated with reduced plasma levels of low density lipoprotein (LDL)-cholesterol (C). To define the contributions of other sequence variations in *PCSK9* to inter-individual differences in plasma levels of LDL-C, we performed large-scale sequencing and genotyping (113 SNPs; density 1 SNP per 500 bp) in an ethnically diverse population-based sample (n=3,543). A panoply of sequence variations with a range of frequencies (<1% to 45%), and various magnitudes of effects on plasma LDL-C level (from increasing by 10% to decreasing by 40%) that were often ethnic-group specific were identified. Neither sequencing alone nor high density SNP analysis alone revealed all the sequence variations at *PCSK9* contributing to variation in plasma LDL-C levels. One rare and two common missense mutations, as well as one common noncoding SNP, were found to be significantly and reproducibly associated with differences in plasma LDL-C levels. The presence of multiple alleles in *PCSK9* associated with increased as well as decreased LDL-C levels suggests that this gene is very sensitive to genetic variation. *PCSK9* provides a model system for the development of a comprehensive gene-focused approach to capture the genetic contribution to a complex quantitative trait.

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CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *K.P. Magnusson[1], S. Duan[2,3,4], H. Petursson[1], H. Sigurdsson[5,6], Z. Yang[2,3], Y. Zhao[2,3], P.S. Bernstein[2], J. Ge[4], F. Jonasson[5,6], E. Stefansson[5,6], G. Helgadottir[5,6], T. Jonsson[1], T. Thorlacius[1], G. Thorleifsson[1], A. Kong[1], H. Stefansson[1], J.R. Gulcher[1], K. Zhang[2,3], K. Stefansson[1].* 1) deCODE genetics Inc. Reykjavik, Iceland; 2) Dept. of Ophthalm and Vis Sci, Moran Eye Center, Univ of Utah; 3) Eccles Inst of Human Genetics, Univ of Utah, Salt Lake City, Utah, US; 4) Zhongshan Ophthalmic Center, Sun Yat-sen Univ, Gaung Zhou, China; 5) Dept. of Ophthalm, National Univ Hospital, Iceland; 6) Faculty of Medicine, Univ of Iceland.

Age-related macular degeneration (AMD) is the most common cause of irreversible visual impairment in the developed world. The two forms of advanced AMD, geographic atrophy (GA) and exudative AMD (Exu), represent different pathological processes in the macula that lead to loss of central vision. Soft drusen, characterized by deposits in macula without visual loss are considered a precursor of advanced AMD. Recently, it has been proposed that a common missense variant, Y402H, in the Complement Factor H (CFH) gene increases the risk for advanced AMD. However, its impact on soft drusen, GA, or exudative AMD or the relationship between them is unclear. We genotyped 581 Icelandic patients with advanced AMD (278 Exu, 203 GA, and 100 with mixed Exu/GA), and 435 with early AMD (of whom 220 had soft drusen). A second cohort of U.S. patients from Utah with advanced AMD (244 Exu and 78 GA) and 109 early AMD cases with soft drusen, were analyzed. We confirmed that the CFH Y402H variant shows significant association to advanced AMD in Iceland (OR 2.39, p-value = 5.9×10-12) and U.S. patients from Utah with advanced AMD (OR 2.14, p-value 2.0×10-9). Furthermore, we show that the Y402H variant confers similar risk of soft drusen as to both advanced forms of AMD (GA or Exu). Soft drusen occur prior to progression to advanced AMD and represents a histological feature shared by Exu and GA. Our results suggest that CFH is a major risk factor for soft drusen, and additional genetic factors and/or environmental factors may be required for progression to advanced AMD.

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Haplotype-Based Association of a Type VII Adenylyl Cyclase Gene Polymorphism with Depression. *L.M. Hines[1], B. Tabakoff[1], L. Saba[1], A. Kaiser[1], B. Grant[3], L. Martinez[1], L. LeGault[2], M. Dongier[2], P. Hoffman[1].* 1) Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO; 2) Department of Psychiatry, McGill University, Montreal, Quebec, Canada; 3) Division of Epidemiology, NIAAA, Rockville, MD.

Adenylyl cyclase is an enzyme that can regulate the physiologic effects of numerous drugs and hormones through the production of cyclic adenosine-3,5-monophosphate (cAMP). Reduced activity of the AC/cAMP signaling system has been implicated in the etiology of major depression. Previous work has shown low adenylyl cyclase activity in platelets and postmortem brain tissue of depressed individuals, and has indicated the heritability of platelet adenylyl cyclase activity. The Type VII isoform of adenylyl cyclase is a major isoform expressed in platelet precursor cells. In a population of 503 Caucasian subjects recruited from Montreal, Canada as part of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence, we have demonstrated a significant association, in women, between a tetranucleotide repeat polymorphism (the 7-repeat allele) in the 3'-untranslated region of the adenylyl cyclase 7 gene (ADCY7), and the phenotype of familial depression (history of DSM-IV depression in the subject and in a first-degree relative). A haplotype in the ADCY7 gene, which contains the repeat polymorphism, was also identified and found to be associated with a significantly higher risk (relative risk 5.1 for homozygotes and 2.1 for the heterozygotes) for familial depression in women. The microsatellite marker alone and the high-risk haplotype, were associated with a similar increased risk for familial depression, suggesting that the microsatellite marker may functionally contribute to familial depression in women, possibly through an effect on transcription or translation of adenylyl cyclase 7. Given the heterogeneity of depression, and the variable nature of response to antidepressant treatments, the presence of the ADCY7 repeat polymorphism may be useful for defining a subtype of major depressive disorder. We are currently investigating the potential role of other AC/cAMP pathway genes.

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Evidence for association/linkage disequilibrium of DNA sequence variants with schizophrenia in the 3 region of neuregulin 1. *D.B. Wildenauer[1], B. Hoefgen[4], M. Albus[5], M. Borrmann-Hassenbach[5], M. Trixler[6], W. Maier[4], S.G. Schwab[2,3].* 1) CCRN, School of Psychiatry, UWA, Claremont, WA, Australia; 2) Centre for Medical Research, UWA, Perth, WA, Australia; 3) Western Australian Institute for Medical Research, Nedlands, WA, Australia; 4) Dept of Psychiatry, University of Bonn, Germany; 5) Mental State Hospital, Haar, Germany; 6) Dept. of Psychiatry, University of Pecs, Hungary.

Schizophrenia is considered to be a complex genetic disorder with several susceptibility genes contributing to its etiology. Association/linkage disequilibrium of DNA sequence variants with schizophrenia has been reported in the last few years for a number of candidate genes. Cumulative evidence has been obtained for neuregulin1, a plausible candidate involved in key neurodevelopmental processes in the central nervous system. The gene extends over more than 1Mb of genomic sequence. We have analyzed the exon dense 3 region of the gene, which extends over 160kb, in a sample of 125 trios with offspring affected with schizophrenia. We used 13 SNPs located in an average distance of 12.2kb. Transmission disequilibrium test revealed nominally significant P-values (0.05) for the three SNPs rs2466061, rs6988339, and hCV2870393. Haplotypes were constructed using the program FAMHAP. P-values of 0.0016 and 0.0008 were produced for a two-marker- and a three-marker haplotype, respectively. The markers are located approximately 1Mb distal from the previously reported (Stefansson et al AJHG 2002,72:83) and replicated association results, but in the same area as two of the markers for which association with schizophrenia has been reported recently by Yang et al (Mol Psychiatry 2003,8:706).

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Detection of a variant in the DRD2 gene involved in schizophrenia using a phylogeny based method. *C. Bardel*[1], *C. Dubertret*[2], *E. Génin*[1], *P. Darlu*[1]. 1) INSERM U535, Villejuif, France; 2) INSERM U676, Faculté Xavier Bichat, Paris, France.

The gene coding for the D2 dopamine receptor (DRD2) is considered as one of the most relevant candidate genes in schizophrenia. Various studies have already been performed on this gene but they lead to conflicting results. In this study, we analyze a sample composed of 103 patients with DSM-IV criteria of schizophrenia and their two parents previously described by Dubertret et al. (2004). The individuals are genotyped for 8 markers (7 SNPs and 1 microsattellite) in the DRD2 region (either in the DRD2 gene or in the X-kinase gene, located downstream). The data are analyzed by a new method described in Bardel et al (2005) which is based on the reconstruction of the evolutionnary history of the haplotypes formed by the 8 markers through a phylogenetic tree. This method consists in looking for groups of haplotypes on the phylogenetic tree that contains an excess of case haplotypes. The polymorphisms defining these groups are likely to be susceptibility loci for the disease. Using this method, we identify the TaqI A1/A2 polymorphism, located in the exon 8 of the X-kinase gene as a potential susceptibility site for schizophrenia, thus confirming the results previsously published by Dubertret et al (2004).

Bardel et al, BMC Genet. 2005;6(1):24

Dubertret et al, Schizophr Res. 2004;67(1):75-85.

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Evidence for sex-specific transmission of DNA sequence variants in the dysbindin gene to offspring affected with schizophrenia. *S.G. Schwab[1,2,6], T. Becker[3], M. Knapp[3], M. Albus[4], M. Borrman-Hassenbach[4], W. Maier[5], D.B. Wildenauer[6].* 1) WAIMR, University of Western Australia,Nedlands, WA, Australia; 2) Centre for Medical Research, UWA, Perth, Australia; 3) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany; 4) Mental State Hospital, Haar, Germany; 5) Dept of Psychiatry, University of Bonn, Germany; 6) CCRN, School of Psychiatry and Clinical Neurosciences, UWA, Perth, Austrlia.

Genetic vulnerability for schizophrenia has been demonstrated consistently in family-, twin- and adoption studies. One of the regions that might harbour susceptibility genes for schizophrenia is a region on chromosome 6p. We have been able to confirm the recently reported association of schizophrenia (Straub et al, AJHG 2002,71:337) with six single nucleotide polymorphisms from intronic regions of the dystrobrevin binding protein (DTNBP1) located in 6p22 in a chromosome 6p linked family sample (73 families with both parents and at least two affected offspring from Germany), as well as in an additional sample of small families comprising 125 parents and a single affected offspring (Schwab et al, AJHG 2003,72:185). Here we report analysis of these DNA sequence variants in the combined family sample for sex-specific transmission. A haplotype composed of rs1011313 (m2), rs760761 (m4), and rs1018381 (m6) produced a P-value of 2.09×10^{-6} for maternal transmissions which is an order of magnitude lower than the previously reported P-value (2×10^{-5}) for the marker combination m2 and m4. Parental asymmetry test for imprinting revealed $P = 4.82 \times 10^{-3}$ for the haplotype with markers m4 and m6. Gender differences in prevalence, age of onset, psychopathology, course, and outcome are well known to be present in schizophrenia and assumed to be due to genetic and/or environmental factors. Our data suggest a contribution of imprinting to these differences.

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Progressive supranuclear palsy is associated with a conserved regulatory region in intron 0 of the tau gene. *S. Melquist[1], R. Rademakers[2], M. Cruts[2], M. Baker[1], R. Crook[1], J. Adamson[1], J. Crook[3], D. Dickson[4], G. Schellenberg[5,6], M. Hutton[1], C. Van Broeckhoven[2].* 1) Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; 2) Department of Molecular Genetics, University of Antwerp, Antwerpen, Belgium; 3) Biostatistics Unit, Mayo Clinic College of Medicine, Jacksonville, FL; 4) Department of Pathology, Mayo Clinic College of Medicine, Jacksonville, FL; 5) Department of Psychiatry and Genetics, University of Washington, Seattle, WA; 6) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA.

Two extended haplotypes exist across the tau gene (*MAPT*) - H1 and H2 - with the H1 haplotype associated with increased risk of progressive supranuclear palsy (PSP). We investigated whether risk for PSP could be narrowed down to a small region or a specific variant on the H1 haplotype. Using 15 haplotype tagging SNPs (htSNPs), capturing more than 90 % of the *MAPT* haplotype diversity, we performed association analysis in a cohort of 274 predominantly pathologically-confirmed PSP patients and 424 matched control individuals. In control individuals, we identified two ancestral extended H1 subhaplotypes, H1A and H1B that accounted for 39% of H1 haplotype diversity. We found that PSP risk is associated with H1B: increasing from 14% in controls to 22% in PSP patients ($p < 0.001$). Analysis of young PSP patients allowed us to narrow the PSP risk on H1B to a specific 22 kb region in intron 0 of *MAPT*. The association with H1 variants within intron 0 was replicated in a second PSP patient - control set. However, our analysis showed that H1 risk variants alone could not explain the overall differences in H1 and H2 frequencies in PSP cases and controls, not even in young patients. Therefore, both specific risk variants on H1 and the protective effect of H2 contribute to the overall population risk for PSP.

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Investigating the Role of SLC6A4 in Response to the Antidepressant Citalopram Utilizing a Large Clinical Sample. *J.B. Kraft[1], E.J. Peters[1], S.L. Slager[2], G.D. Jenkins[2], M.S. Reinalda[2], P.J. McGrath[3], S.P. Hamilton[1].* 1) University of California, San Francisco; 2) Mayo Clinic College of Medicine; 3) New York State Psychiatric Institute and Columbia University.

The serotonin transporter is the target of many antidepressants, and the gene (SLC6A4) encoding this protein has been tested for association with phenotypes such as major depressive disorder (MDD) and response to selective serotonin reuptake inhibitors (SSRIs). Many studies have reported positive associations while others report no association. In previous studies utilizing a patient population with MDD (N=96) who were taking the SSRI fluoxetine, we failed to detect any association between SSRI response and the oft-studied length polymorphism (HTTLPR)in the promoter region. However, we did detect an association with two additional variants in the 5 region of the gene (rs25531/rs25533). We sought to further test the hypothesis that SLC6A4 is associated with SSRI response by using a larger patient population focusing on these 3 polymorphisms: HTTLPR, rs25531, and rs25533. We genotyped 1,953 subjects with MDD who have all taken the same SSRI, citalopram, as part of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study to investigate the role of SLC6A4 in SSRI response.

Genotyping was performed using RFLP analysis for the 2 SNPs and agarose gel separation for the HTTLPR. Single locus and haplotype analyses among the 3 markers were performed. Our analysis showed no association between the 3 markers and response to SSRIs (P values all >0.05), adjusting for ethnicity. A global score test of the association between 3-locus haplotypes and response was not significant (P=0.27). We observed extensive linkage disequilibrium in this 2kb region (d=0.93-0.99). These results fail to replicate findings showing HTTLPR association with SSRI response. Further, with our larger sample, we also failed to replicate our previous findings of association with 2 SNPs (rs25531/rs25533) and SSRI response. Our findings do not support an association between response to SSRIs and DNA variation at the serotonin transporter locus in the largest sample to date.

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Allelic and haplotypic association of NOS2A with type 1 diabetes in Newfoundland. *K. Wang[1], M. Liu[1], B. Bharaj[1], M. Lu[1], H.T. Chen[1], J.A. Curtis[2], L.A. Newhook[2], A.D. Paterson[1,3].* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Pediatrics, Memorial Univ. of Newfoundland, NF, Canada; 3) Dept. of Public Health Sciences, Univ. of Toronto, Canada.

Type 1 Diabetes (T1D) is an autoimmune disease which leads to destruction of insulin producing beta-cells in the pancreas. The NOS2A gene has been proposed to play an active role in this destructive process and might be a susceptibility gene for T1D. However, only one significant association between the T allele at Ser608Leu (exon 16) and T1D in HLA DR3/4-positive affected offspring has been reported (P=0.008, Johannesen et al. 2001). We genotyped six SNPs and (CCTTT)n around the NOS2A gene in 538 mulipex families from a relatively isolated population of Newfoundland (NF) which has a high incidence of T1D. The extended TDT (ETDT) was used to test for association between the markers and T1D. The Evolutionary Tree-TDT (ET-TDT), which takes advantage of grouping haplotypes through a cladistic analysis using their evolutionary relationships, was also carried out to test for excess transmission of haplotypes from parents to affected children. The affected offspring were stratified by the median age of diagnosis (AOD) into two groups: early (AOD <=10) and late (AOD>10). Interestingly, the T allele at Ser608Leu (exon 16) revealed significant transmission disequilibrium from unaffected parents to affected offspring (P=0.0033) in the whole dataset while allele 190 at (CCTTT)n demonstrated significant association to the early group (P=0.0011). Furthermore, one five-SNP haplotype was strongly associated with T1D for the whole dataset and the early group (P=0.0083 and 0.0018, respectively). Moreover, cladistic analysis confirmed that the same five-SNP haplotype was significantly associated with T1D both in the whole dataset and the early group (P=0.0026 and 0.0019, respectively). In addition, two clades based on the haplotypes inferred from (CCTTT)n and its two flanking SNPs were significantly associated with T1D in the early group (P=0.0016 and 0.0009, respectively), whereas only weak association was found using ETDT. In conclusion, our findings provide evidence for a role of the NOS2A gene in T1D in NF.

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Association analysis of a LINE1 polymorphism in the gamma-aminobutyric acid A receptor, gamma 1 gene (GABRG1) in autism. *S. Horike[1], S. Choufani[1], J. Vincent[1, 2], S.W. Scherer[1].* 1) Department of Genetics and Genomic Biology, University of Toronto, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Autistic disorder (AD) is a neuro-developmental disease characterized by abnormalities in social, communicative, and behavioral functioning. We report here a family with two brothers diagnosed with AD each having inherited a paracentric inversion of the short arm of the chromosome 4 [46, XY, inv(4)(p12-p15.3)]. Molecular and cytogenetic analysis revealed that this inversion is inherited from the mother who inherited it from her father, neither of whom showed obvious symptoms. The proximal breakpoint (4p12) mapped within intron 7 of the gamma-aminobutyric acid A receptor, gamma 1 (GABRG1). Interestingly, we also discovered a LINE1 polymorphism located in the same intron of GABRG1. Studies have shown that LINE1 insertions can affect expression by acting as a 'gmolecular rheostat'h of target genes. Therefore, we hypothesized that this intron 7 LINE1-element may influence the level and timing of GABRG1 expression in other autism patients. Initially, we are investigating this hypothesis by testing the frequency of the LINE1 allele in autism patients and controls. Our preliminary data indicates the allelic frequencies in the autism patients (n=298) vs. control (n=182) to be: +/+ (34.9%; 25.8%), +/- (45.3%; 48.3%), -/- (19.8%; 25.8%), respectively. We are currently expanding these studies in a wider autism and control dataset (including family-based controls) and completing functional studies.

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PRKCA shows association to multiple sclerosis in two populations and is flanked by elements of duplication superstructure. *J. Saarela[1], D. Chen[2], S.P. Kallio[1], A. Montpetit[3], E. Choi[2], T. Miettinen[5], R. Asselta[1], D. Bronnikov[1,2], M. Lincoln[4], A. Palotie[5], G.C. Ebers[4], T.J. Hudson[3], L. Peltonen[1,6].* 1) Dept Molecular Medicine, Natl Public Health Inst, Helsinki, Finland; 2) Dept Human Genetics, UCLA Medical School, Los Angeles, CA, USA; 3) McGill Univ and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Dept Clinical Neurology, Radcliffe Infirmary, Univ of Oxford, Oxford, UK; 5) Finnish Genome Center, Univ of Helsinki, Helsinki, Finland; 6) Dept Medical Genetics, Univ of Helsinki, Helsinki, Finland.

We have established linkage in Finnish MS families to 17q24, and identified initial association to the PRKCA gene. The gene with the 1 Mb flanking regions was explored in detail in two, large independent MS cohorts. A SNP haplotype analysis revealed an allelic variant of PRKCA being significantly over-represented in Finnish and Canadian MS cases (Odds Ratio 1.34, 95th CI 1.13-1.59). The expression level of PRKCA correlates with the copy number of the PRKCA risk allele, implying its biological relevance. Though the critical role of PRKCA in regulating immune responses makes it a strong candidate for MS susceptibility gene within this interval, we also observed some evidence of association to MS with SNPs mapping telomeric to PRKCA, within the interval flanked by duplications. To evaluate whether the SNPs associated with MS could be in LD with an yet unidentified chromosomal rearrangement polymorphism affecting several genes within the locus, we explored the complex structure of 17q in more detail. By blasting the exons of the genes mapping to the duplications at 63Mb and 66Mb on 17q, we observed highly homologous sequences to reside 13 times within 17q. A cross-species genome comparison revealed that the human and chimpanzee genomes have similar but not the identical duplication patterns, while the mouse genome has only one copy of the sequence. A retrotransposon-like element mapping almost exclusively to 17q was found in all copies of the superstructure, stimulating the hypothesis that the element might have contributed to the complex structure of 17q and can predispose to chromosomal rearrangements.

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Novel Microdeletions and Microduplications in Subjects with an Autism Spectrum Disorder (ASD) Identified using 1 Mb Array-Comparative Genomic Hybridization (array-CGH). *C. Harvard[1,5], M. Koochek[2,5], Y. Qiao[1,5], C. Fawcett[1], P. Malenfant[3,4,5], SC. Creighton[2,5], MJ. Hildebrand[2,5], L. Armstrong[2], B. McGillivray[2], P. MacLeod[2], M. VanAllen[2], JJA. Holden[3,4,5], E. Rajcan-Separovic[1,5], MES. Lewis[2,5].* 1) Depts. of Pathology &; 2) Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Depts. of Physiology &; 4) Psychiatry, Queen's University, Kingston,Canada; 5) ASD-CARC; www.autismresearch.ca.

ASDs are a group of neurodevelopmental disorders characterized by significant social, communication and behavioural impairments affecting 1/160-1/250 persons. Although current understanding of the complex genetic mechanisms underlying ASDs is limited, sub-microscopic/sub-telomeric chromosomal abnormalities have been reported in approximately 10% of individuals. Whole genome array-CGH is a powerful tool for identifying submicroscopic deletions and duplications undetectable by conventional cytogenetic techniques. We are using 1 Mb array-CGH (Spectral Genomics)to determine the prevalence of cryptic subchromosomal changes in subjects with an ASD. Four cases with known abnormalities, including mosaic and non-mosaic gain of 15q11-q13, del(22)(q13) and t(5,7)(p15.1p12.2) with inv(3)(p24q24), were used as positive controls. Twenty-two cases with normal high resolution karyotypes were studied. The del(22) and gains of 15q were confirmed via array-CGH. The case with structural rearrangements of chromosomes 3, 5 & 7 revealed 3 novel microdeletions undetectable by routine cytogenetic analysis. Of the karyotypically normal cases, 4 had novel submicroscopic changes. Two cases had submicroscopic imbalances not detected in unaffected family members (del of 2p in Case 1 & dup of proximal 15q/microdeletion of 14q in Case 2) as well as inherited rearrangements (del of Xp in Case 1 and dup of 6q in Case 2). In Cases 3 and 4 unique single clone changes were noted and their origin is under investigation. This pilot study identified novel cryptic subchromosomal changes in 18% of probands with an ASD, suggesting that the co-occurrence of autism with submicroscopic chromosomal abnormalities is significant and will be useful for localizing candidate gene regions for autism.

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Gene Expression in Intracranial Aneurysm. *S. Weinsheimer[1], M. van der Voet[1], G.M. Lenk[1], M. Skunca[1], A. Ronkainen[2], H. Kuivaniemi[1], G. Tromp[1].* 1) CMMG, Wayne State University, Detroit, MI; 2) Department of Neurosurgery, University of Kuopio, Kuopio, Finland.

Frequently candidate regions from linkage analyses contain several hundreds of genes. An approach to reduce the list of positional candidate genes is to use gene expression data from diseased and healthy tissue. Genes that are not expressed, either in diseased or healthy tissue, may be excluded from the candidate list. Here we used this approach to assist in the identification of candidate genes for the complex disease intracranial aneurysm (IA). IAs may grow, rupture, and lead to subarachnoid hemorrhage. Each year about 30,000 individuals in the United States suffer ruptured IA. Despite evidence for a genetic predisposition for IA and previously reported linkage, including to chromosome 19q13, specific genes that contribute to IA have yet to be identified. **Methods**: Intracranial arteries from individuals with IA were obtained post-mortem and pooled (n=4) into sample sets to represent aneurysmal intracranial arteries and healthy contralateral vessels (controls) obtained from the same patients. Five pools of total RNA were amplified and hybridized to Affymetrix HU133 Plus 2.0 GeneChips. Samples were from aneurysms of the anterior communicating- and middle cerebral-artery, and from healthy intracranial arteries. Data were analyzed using the MAS 5.0, gcRMA, and RMA methods to identify expressed genes. **Results**: Several chromosome 19q13 positional and functional candidate genes were expressed in intracranial arteries. Some are known to be involved in important processes including extracellular matrix metabolism, apoptosis, and inflammatory response. A subset of the expressed positional candidate genes include: PLAUR, APOE, CARD8, HIF3A, NFKBIB, NOSIP, and several in the kallikrein gene cluster: KLK3, -4, -7, -13, and -15. **Conclusion**: These data suggest a role for kallikreins in the pathophysiology of IA. These gene expression data may be useful also for other diseases affecting intracranial arteries. Gene expression analysis is a useful approach for reducing a list of positional candidate genes.

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Are Common Variants in APP Risk Factors for Late-onset Alzheimers Disease? *P. Nowotny[1], X. Simcock[1], A.L. Hinrichs[1], S. Smemo[1], J.S.K. Kauwe[1], S. Cherny[1], J. Morris[2], A. Goate[1,2].* 1) Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Neurology, Washing Univ Sch Medicine, St Louis, MO.

Linkage studies have suggested a susceptibility locus for late-onset Alzheimers disease (LOAD) on chromosome 21. A functional candidate gene in this region is the amyloid precursor protein (APP). Previously, coding mutations in APP have been associated with early onset Alzheimers disease (EOAD) and triplication of APP is associated with AD pathology in Downs Syndrome, suggesting that over-expression of APP may be a risk factor for LOAD. Although APP is a strong functional and positional candidate, to date there has been no thorough investigation using a dense map of SNPs across the entire APP gene. In order to thoroughly investigate the role of APP in the risk of LOAD, we genotyped more than 42 intronic SNPs, spanning 300 kb in and around APP. The SNPs were genotyped in a LOAD case-controls series of 270 cases and 270 controls from the ADRC at Washington University. We found significant associations with SNPs mainly in the region between introns 2 and 3, with rs2000989 (p-value= 0.0106) being the most significant. However, haplotypes including these SNPs showed no increase in association compared to the individual SNPs. We also stratified for presence or absence of the APOE 4 allele because the linkage to chromosome 21 was observed in the sibling pairs without an APOE 4 allele. This stratified analysis did not reveal a consistent trend in either stratum. We are currently genotyping SNPs in introns 2 and 3 in two independent case-controls samples and a case-control series derived from our linkage sample to try to replicate these findings.

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Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *J.S. Sutcliffe[1], R.J. Delahanty[1], H.C. Prasad[2], J.L. McCauley[1], Q. Han[2], L. Jiang[1], C. Li[1], S.E. Folstein[3], R.D. Blakely[2].* 1) Center for Human Genetics Research, Dept of Mol Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) Center for Molecular Neuroscience, Dept of Pharmacology, Vanderbilt University, Nashville, TN; 3) Dept Psychiatry, Johns Hopkins University, Baltimore, MD.

Autism is a spectrum of neurodevelopmental disorders with a primarily genetic etiology exhibiting (1) deficits in development of language and (2) social relationships, as well as (3) patterns of repetitive, restricted behaviors or interests, and resistance to change. Elevated platelet serotonin (5-HT) in 20-25% of cases, and efficacy of selective 5-HT reuptake inhibitors (SSRIs) in treating anxiety, depression, and repetitive behaviors points to the 5-HT transporter (SERT, 5-HTT) as a strong candidate. Association studies involving the functional insertion/deletion polymorphism in the promoter (5-HTTLPR) and a polymorphism in intron 2 are inconclusive, possibly due to phenotypic heterogeneity. Nonetheless, mounting evidence for genetic linkage of autism to the chromosome 17q11.2 region harboring the SERT locus (SLC6A4) supports a genetic effect at or near this gene. We confirm recent reports of sex-biased genetic effects in 17q by showing highly significant linkage (HLOD=8) driven by families with only affected males. Association to common alleles fails to explain observed linkage, therefore we hypothesized that preferential transmission of multiple alleles does. From 120 families most contributing to linkage at 17q11.2, we found 4 coding substitutions at highly conserved positions and 15 other variants in 5' noncoding and other intronic regions transmitted in families exhibiting increased rigid-compulsive behaviors. One coding variant, shows an increased frequency, substantial deviation from Hardy-Weinberg equilibrium, elevated transporter activity, and is refractory to PKG and p38 MAP kinase-mediated regulation. In the aggregate, these variants show significant linkage and association to autism. Our data provides strong support for a collection of multiple, often rare, alleles at SLC6A4 as imposing risk for autism.

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Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene-environment interactions. *A.L. Boyles[1], A.V. Billups[1], D.G. Siegel[1], S.H. Slifer[1], M.C. Reed[2], H.F. Nijhout[3], T.M. George[4], A.G. Bassuk[5], J.A. Kessler[5], J.R. Gilbert[1], M.C. Speer[1], NTD Collaborative Group[1].* 1) Ctr Human Genetics, Duke Univ Med Ctr, Durham, NC; 2) Dept Mathematics, Duke Univ, Durham, NC; 3) Dept Biology, Duke Univ, Durham, NC; 4) Dept Surgery, Duke Univ Med Ctr, Durham, NC; 5) Northwestern Univ Feinberg School of Med, Chicago, Illinois.

Folate metabolism pathway genes have been examined for association with neural tube defects (NTDs) due to the known ability of folic acid to reduce the risk of this birth defect. Studies of polymorphisms in these genes usually look at them individually often producing conflicting results. In 318 Caucasian American families with lumbosacral myelomeningocele we examined 10 folate transport and metabolism genes: 5,10 Methylenetetrahydrofolate Reductase (MTHFR), Methionine Synthase (MTR), Methionine Synthase Reductase (MTRR), Reduced Folate Carrier 1 (RFC-1), Cystathionine Beta-sythase (CBS), Methylenetetrahydrofolate Dehydrogenase 1 (MTHFD-1), Serine Hydroxymethyltranferase (SHMT), Folate Receptor Alpha (FolR), and Folate Receptor Beta (FolR). While no SNPs was significant singly using family-based association methods, combinations of 3 and 4 SNPs were significant using Extended Multifactor-Dimensionality Reduction (EMDR). The thermolabile C677T polymorphism in MTHFR was only significant in combination with two polymorphisms in CBS (p=0.01). Mathematical models of the folate pathway [Nijhout et al., 2004] show that reduced activity of MTHFR and CBS greatly increase homocysteine levels, but not in a synergistic manner. If the associated polymorphisms lower the efficiency of the pathway such that homocysteine levels rise above a threshold level, we would expect a similar effect from other folate pathway polymorphisms. More thorough interrogation of these genes as well as environmental influences on folate metabolism are needed. With the inclusion of maternal folate supplementation data, this study provides valuable insight into the significance of gene-gene as well as gene-environment interactions in the folate pathway and their role in neural tube defects.

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Haplotypes over the APP gene that influence risk of Alzheimer's disease in women. *T. Jonsson[1], T. Thorlacius[1], H. Petursson[1], S. Bjornsson[2], P.V. Jonsson[2], J. Snaedal[2], A. Levey[3], A. Kong[1], J. Gulcher[1], K. Stefansson[1].* 1) Decode Genetics, Reykjavik, Iceland; 2) Landspitali University Hospital, Geriatric Department, Reykjavik, Iceland; 3) Emory University, Department of Neurology, Atlanta, GA.

Humans that reach extreme age have done so in part by avoiding or delaying the onset of Alzheimers disease (AD). Therefore, it is expected that the frequency of protective AD variants be greater in cognitively intact longevous individuals than in the general population.

We searched for such variants using linkage analysis in the Icelandic population. A locus for human longevity with a multipoint non-parametric LOD score of 2.4 on Chromosome 21 was identified in families containing cognitively intact longevous individuals. The gene encoding the Amyloid Precursor Protein (APP) resides within the linkage region. Haplotype analysis of single nucleotide polymorphisms (SNPs) within the APP gene reveals a haplotype conferring protection against AD in these longevous individiduals (allelic frequency 10.6% vs 4.9% in AD cases, nominal p-value 4.010^{-6}). Furthermore, a common at-risk haplotype for Alzheimers disease was identified (allelic frequency 42.4% vs. 34.9% in cognitively intact controls; p-value 7.010^{-4}). The observed effects appear to be largely female-specific, with an Odds Ratio (OR) for female heterozygous carriers near 1.7 (p-value 5.310^{-6}) and 3 (p-value 1.410^{-6}) for the at-risk and protective haplotypes, respectively. Expression analysis in human lymphoblastoid cell lines shows that homozygous carriers of the at-risk haplotype for AD have an isoform-independent decreased expression of APP.

Most studies of genetic variations in APP reported to date have focused on mutations in the open reading frame of the gene leading to mendelian phenocopies of AD. Our findings indicate that genetic variants in the APP gene play an important role in the development of the common, late onset, form of AD.

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Haplotype analysis of the Androgen Receptor in Male Pattern Baldness using HapMap tag-SNPs. *J.A. Ellis, K.J. Scurrah, S.B. Harrap.* Department of Physiology, The University of Melbourne, Australia.

Androgenetic alopecia, or male pattern baldness (MPB), is a strongly heritable complex phenotype that requires the presence of androgen for expression. In 2001, we published the first evidence of a strong association between a synonymous SNP (*Stu*I RFLP or rs6152) in exon 1 of the Androgen Receptor (*AR*, X chromosome), and MPB[1]. This association has since been confirmed by two independent studies[2,3]. The SNP lies between two triplet repeat polymorphisms shown to have functional significance, however neither of these polymorphisms appear to be independently associated with MPB in our population. Sequencing of all exons, untranslated regions, and regulatory regions totalling 7 kb upstream of *AR* failed to reveal any polymorphisms associated with MPB. We used HapMap to determine patterns of linkage disequilibrium (LD) spanning over 500 kb in and around *AR*, and found almost all HapMap SNPs were contained in two strong LD blocks. We selected four tag-SNPs (LD block 1: rs4827542, rs 5964602; LD block 2: rs1415270, rs4827405), that defined all haplotypes observed in the HapMap data. These SNPs were genotyped in 56 cases (men aged 30 years with cosmetically significant baldness) and 107 controls (men aged 50 years with no evidence of baldness). Single SNP, and haplotype association analyses were performed using the Haploview program. Of the single SNPs, rs4827542, located more than 200kb upstream of *AR*, was the most strongly associated with MPB (T allele: cases 96% vs controls 72%; P = 0.0003). The most commonly identified haplotype was detected in 93% of cases, but only 65% of controls (P = 0.00004). In conclusion, single SNP association analyses suggest that more strongly associated SNPs may lie a significant distance upstream of *AR*, and that tag-SNP haplotype analysis better defines cases and controls than single SNPs. However we have yet to identify the functional variant(s) in *AR* that are contributing to MPB, which may lie some distance from the coding sequence.

[1] Ellis JA et al. J Invest Dermatol; [2] Hayes VM et al. Cancer Epidemiol Biomarkers Prev 2005;14:993; [3] Hillmer AM et al. Am J Hum Genet 2005;17:140.

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Genomic studies of *FCGR3B* in SLE. *E. Petretto[1], C. Roberton[1], T. Aitman[2], T. Cook[1], T. Vyse[1].* 1) Imperial College, London, UK; 2) MRC CSC, London, UK.

Introduction: SLE is an autoimmune disease and complex genetic trait. The clinical manifestations include immune complex mediated glomerulonephritis. Linkage analyses in SLE have mapped a region around chromosome 1q23. Association studies have implicated functional polymorphisms in three of five IgG receptors that are encoded in tandem within this region. We have already shown that *FcR2A-H131R* and *FcR3A-F158V* act in concert to slightly increase the risk of SLE (submitted). In this study we have focused attention on the least studied member of this gene complex, *FCGR3B*. **Methods:** Patients with SLE and their siblings were taken from a UK SLE nuclear family collection. The study was ethically approved MREC 98/2/6. Quantitative real-time (qRT)-PCR was carried out using SYBR Green to estimate copy number at *FCGR3B*. To calculate the overall risk/protection associated with *FCGR3B* the data set were fitted with logistic regression model by SPSS 12.0. **Results:** 375 European nuclear SLE families were genotyped at two polymorphisms in the *FCGR3B* gene: one in the promoter and the second encoding the NA1/NA2 allotypes. In contrast with markers typed in the *FCGR2A* and *3A* genes, both *FCGR3B* markers generated significant Mendelian errors (n = 63) and deviated from HWE. Using these families we tested the hypothesis that the median copy number deviated significantly from the expected value in a diploid genome. This was rejected for the single-copy control, *CD36* ($p = 0.25$), but was significant for *FCGR3B* ($p = 0.0004$). We examined the *FCGR3B* copy number in all patients with nephritis (n = 74) compared with unrelated matched siblings (n = 153). A logistic regression analysis provided support for reduced *FCGR3B* copy number in lupus nephritis ($p = 0.001$, 95% CI 0.001-0.0008) and a stepwise model confirmed that *3B* copy provided a statistically reliable model ($^2 = 7.6$, $p = 0.006$) whereas the inclusion of *FcR2A-H131R* and *3A-F158V* produced poorer models. **Conclusion:** Reduced copy number of *FCGR3B* confers a greater risk to lupus nephritis than other associated polymorphisms at the *FcR* locus. This may reflect a role for neutrophils Fc receptors in immune complex removal from the glomerulus.

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Association between *PADI4* and rheumatoid arthritis: a mata analysis. *K. Ikari, M. Kuwahara, T. Nakamura, S. Momohara, M. Hara, H. Yamanaka, T. Tomatsu, N. Kamatani.* Institute of Rheumatology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.

Recently, the peptidylarginine deiminase type 4 gene (*PADI4*) was reported to be associated with rheumatoid arthritis (RA) in a Japanese population. The strongest association was observed for a single nucleotide polymorphism (SNP) located in intron 3 of *PADI4* (P = 0.000008). In a subsequent study, we confirmed the result with the large series of case-control samples using the same genetic markers within the same ethnic background as the original study (P = 0.0008). Despite the strong association results observed in a Japanese population, Barton et al. failed to replicate the observed association in a United Kingdom population. Our objective was to determine whether a reported association is caused by a common variance through different ethnicities, using a meta-analysis combining the results of three association studies from two different ethnic groups.

Meta-analysis was performed under a recessive model, assuming minor-homozygote versus other genotypes, as indicated in the original Japanese study. Since padi4_104 was the only SNP used in all three studies it was included in the meta-analysis. The Breslow-Day test for homogeneity was calculated and conditional independence was assessed using the Mantel-Haenszel test. The common odds ratio with a 95% confidence interval (CI) was also calculated using the Mantel-Haenszel method.

Meta-analysis confirmed the association (P = 0.0002). Common odds ratio calculated with the Mantel-Haenszel method was 1.40 (95% CI = 1.17 - 1.67). The result of a meta analysis provides strong support for the role of *PADI4* in RA. To conclude, we suggest that the association between *PADI4* and RA is attributed to a common variance, irrespective of ethnicity. More powerful validation studies of other ethnic populations are warranted to support this conclusion.

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Association and interaction of the IL4, IL13, IL5, IL3, IL12B, IL9, CD14 and IL4R genes with asthma in Chinese population. *X. Kong, H. Zhang, L. Wang, H. Chen, W. Huang, L. Zhang, L. Hu.* Health Sci Center, Sibs, Shanghai, Shanghai, China.

Cytokines, having central functions in immunological and inflammatory process, are always expected to play important roles in the pathogenesis of various diseases, such as asthma. Genetic polymorphisms of those cytokine and cytokine receptor genes are the focus of genetic association studies. In an effort to identify gene(s) whose variant(s) are associated with the asthma, we screened all exons and their flanking regions, as well as the promoter region (1.5kb) of eight genes, including IL4, IL13, IL12B, IL5, IL3, IL9, CD14 and IL4R.We could identify 42 single nucleotide polymorphisms, 15 of which were novel. Then, we examined the genetic effects of 30 single nucleotide polymorphisms in eight cytokine and cytokine receptor genes on asthma in a Chinese asthma cohort (n=537). Genetic association analysis of polymorphisms revealed that six polymorphisms (SNP18685711¡¢SNP18686951¡¢ SNP18686994¡¢ SNP18687043 ¡¢SNP18687051 ¡¢SNP18687259) in IL4R gene, three of which resulted in an amino-acid change, showed significant association with the risk of asthma (P=0.00023). Further analysis indicates that these six polymorphisms segregated in strong linkage disequilibrium. This information about the genetic association of important genes with asthma might provide valuable insights into strategies for the pathogenesis of asthma.

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Evidence that KIAA0319 is a susceptibility Gene for Developmental Dyslexia on Chromosome 6. *J. Williams[1], N. Cope[1], D. Harold[1], G. Hill[1], V. Moskvina[1], J. Stevenson[2], P. Holmans[1], L. Jones[1], M. Owen[1], M. O'Donovan[1].* 1) Psychological Med, University Wales Col Medicine, Cardiff, United Kingdom; 2) Faculty of Medicine, University of Southampton, Southampton, United Kingdom.

Developmental dyslexia, (DD), is a relatively common, complex cognitive disorder that affects 5-10% of school-aged children. Genetic linkage and association studies have implicated a number of chromosomal regions that may harbor susceptibility genes for the disorder. In particular, linkage between DD and chromosome 6p has been replicated in a number of independent samples. By performing a systematic, high-density linkage disequilibrium (LD) screen of genes with publicly available SNPs in the most consistently supported region on 6p, we have previously found evidence that variation in the KIAA0319 gene is associated with increased risk of developing dyslexia (Cope etal, AM.J.Hum.Genet. 76,2005) Logistic regression analysis showed that two SNPs, rs4504469 and rs6935076, best explained DD status (p=0.00001). While the haplotype composed of these two markers is significantly associated with DD, these results do not necessarily imply that these two SNPs are the direct susceptibility variants. As such, we have further analyzed the KIAA0319 gene by resequencing all exons and genotyping the non-redundant polymorphisms identified. In addition we have assessed the LD structure of the gene, and genotyped all tag SNPs within haplotype blocks containing an associated SNP. Additional SNPs in conserved non-coding regions were also typed. In total, 22 SNPs have been genotyped in a case/control sample of 223 subjects with DD and 273 controls, in addition to 4 SNPs typed previously. We report here the refined evidence for involvement of KIAA0319 in susceptibility to developmental dyslexia.

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Test of association of *DTNBP1, NRG1, DAOA/G30, DAO,* and *DRD2* with schizophrenia in two US family samples. *J. Duan, M. Martinez, A.R. Sanders, C. Hou, A. Krasner, D.B. Schwartz, G.J. Burrell, P.V. Gejman.* Center for Psychiatric Genetics, Department of Psychiatry and Behavioral Sciences, ENH Research Institute and Northwestern University, Evanston, IL.

We report here a study of association of *dysbindin (DTNBP1)*, *neuregulin-1 (NRG1), D-amino-acid oxidase activator (DAOA)/G30, D-amino-acid oxidase (DAO)*, and the *dopamine receptor D2 (DRD2)* in a sample of 136 United States (US) schizophrenia families largely of European Ancestry (EA). Besides all the previously positively associated single nucleotide polymorphisms (SNPs), we selected additional SNPs to increase map coverage and to explore for a potential causative SNP by prioritizing for known coding variants. We assessed the data quality by testing the repeatability and reliability of our genotyping methods (Fluorescence-polarization detection (FP), TaqMan, and SNPlex), and by checking for Hardy-Weinberg equilibrium (HWE) departures, Mendelian errors, and unlikely recombinants. For *DTNBP1*, Family-Based Association Test (FBAT) analyses with 11 tag SNPs (r^2>0.8) showed evidence for association between rs7758659 and disease (P=0.004). This actually represents evidence for three highly correlated SNPs including rs7758659 (r^2>0.94), which span about 55 kilo base pairs (kb) of intron 7 containing 3 cryptic exons. The most significant haplotype C-A (P=0.001, global P=0.0015) consists of rs7758659, and the previously most replicated SNP, P1635 (rs3213207). For *DRD2*, the promoter -141C Ins/Del was observed to be associated in the EA subset (P=0.008). Though the synonymous C957T did not show significant association, when combined with Taq1A, the P value of the haplotype C-G reached modest significance (P=0.02, global P=0.07) in which C is the allele that previously was shown to have higher *DRD2* mRNA stability (Duan et al., 2003). Lack of association for the remaining tested genes might reflect disease allelic heterogeneity, inadequate statistical power deriving from sample size, or that previously reported associations actually reflect false-positives. A robust test will require a comprehensive set of markers and larger samples.

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Missense mutation in a bitter taste receptor (hTAS2R16) influences risk for alcohol dependence. *A. Hinrichs[1], J.C. Wang[1], B. Bufe[2], J.M. Kwon[1], J. Budde[1], R. Allen[1], S. Bertelsen[1], W. Evans[3], D. Dick[1], T. Foroud[4], R. Crowe[5], V. Hesselbrock[6], M. Schuckit[7], L. Almasy[8], H. Begleiter[9], B. Porjesz[9], H.J. Edenberg[4], W. Meyerhof[2], L.J. Bierut[1], A.M. Goate[1].* 1) Dept Psychiatry, Washington Univ Sch Med, St Louis, MO; 2) Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; 3) Laboratory of Neurogenetics, National Institute of Aging, National Institute of Health, Bethesda, MD; 4) Indiana University School of Medicine, Indianapolis, IN; 5) University of Iowa School of Medicine, Iowa City, IA; 6) University of Connecticut School of Medicine, Farmington, CT; 7) UCSD School of Medicine, La Jolla, CA; 8) Southwest Foundation, San Antonio, TX,; 9) SUNY Health Science Center at Brooklyn, Brooklyn, NY.

The aim of the Collaborative Study of the Genetics of Alcoholism (COGA) is to find genes that modify susceptibility to alcohol dependence and related phenotypes. A linkage finding (LOD=2.9) on chromosome 7q for alcohol dependence is located near a cluster of bitter taste receptors (TAS2Rs). We genotyped three functional single nucleotide polymorphisms (SNPs) in TAS2R16, a gene, which encodes a receptor for beta-glucopyranosides. The non-synonymous SNP rs846664 showed strong association with DSM-IV alcohol dependence (p=0.00018) in the COGA linkage sample and an additional set of trios unrelated to the linkage sample. The risk allele is rare in Caucasians (MAF 0.6%), but is present in 45% of African Americans making it a significant risk factor for alcoholism in this population. The SNP results in an amino acid substitution (N172K) in the extracellular loop between transmembrane domains four and five. In G-coupled protein receptors such as the TAS2Rs this domain has been associated with ligand binding and suggests that this amino acid substitution may alter signaling/taste perception. Our functional assays show that constructs containing the rare allele, K172, exhibit a higher EC50 (half-maximal response) for each of the agonists tested supporting the hypothesis that this SNP is a functional allele that increases risk for alcoholism.

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Identifying candidate coronary artery disease susceptibility genes through genomic convergence. *J.J. Connelly[1], T. Wang[1], A. Dobra[2], J. Rose[1], L. Wang[1], L. Huang[1], B. Pedersen[1], C. Haynes[1], J.M. Vance[1], W.E. Kraus[3], P. Goldschmidt-Clermont[3], E.R. Hauser[1], S.G. Gregory[1], for the GENECARD and AGENDA investigators.* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Institute of Statistics and Decision Sciences, Duke University, Durham, NC; 3) Department of Medicine, Duke University, Durham, NC.

We aim to characterize genes involved in the development of Coronary Artery Disease (CAD) using several different genetic methods. We have identified GATA2 as a transcription factor with an increased potential to be involved with CAD susceptibility. GATA2 is known to regulate hematopoiesis and the development of the cardiovascular system. We obtained evidence for the involvement of GATA2 in CAD from several independent lines of investigation: 1)GATA2 maps beneath our GENECARD (early onset CAD genome scan) linkage peak on Chromosome 3q, which meets the criteria for genome wide significance and, upon stratification, was significant for linkage to both Acute Coronary Syndrome and the absence of diabetes; 2)Analysis of differential gene expression within distal and proximal regions of the aorta identified GATA2 expression patterns as highly predictive of atherosclerosis susceptibility; 3)GATA2 DNA binding sites are significantly over-represented in the upstream region (3kb) of genes that are differentially expressed in atherosclerotic aortas when compared to controls. We further evaluated evidence for a role for GATA2 in CAD in an independent case-control data set including 1,037 subjects with catheterization data from the Duke Coronary Catheterization Lab (the CATHGEN study). We identified four SNPs within GATA2 that are significantly associated (p0.01) with affected status using logistic regression. Two of these SNPs were also significant (p.05) using family-based association methods in the GENECARD study. However,these SNPs do not account for the linkage observed in the GENECARD families. These data suggest a role for GATA2 in CAD as one of many genes increasing susceptibility to CAD. We present our data as a model for the integration of genomic methods to identify candidate disease genes.

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Mutation Screening of Candidate Genes for the X-Linked High-Grade Myopia Locus (MYP1). *N.F. Wasserman[1], P.C. Paluru[1], E.N. Campbell[2], T.L. Young[1,2].* 1) Divisions of Ophthalmology and Genetics, Childrens Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania School of Medicine, Philadelphia, PA.

The first locus (MYP1) for high-grade myopia was mapped to chromosome Xq28 in a large Danish family with myopia and cone photoreceptor dysfunction. We previously performed analysis on a second Danish family also exhibiting high-grade myopia with definitive cone dysfunction, identifying a linked region at Xq27.3-Xq28. Reported mapping studies performed in two Indian pedigrees with X-linked high-grade myopia (no electrophysiology studies conducted) contracted the putative gene interval to 1.2cM at the telomeric end of Xq28. We sought to identify the causative gene(s) in our MYP1 family by direct sequencing of 7 known (CTAG1, CTAG2, CLIC2, H2AFB3, TMLHE, SPRY3 and SYBL1) and 2 hypothetical positional candidate genes in this reduced 1.2 cM region. Reverse-transcription PCR was performed to confirm gene expression in human ocular tissues. Gene sequence was elucidated using public databases. Exonic regions and exon/intron boundaries for all genes were sequenced using genomic DNA from affected, carrier, and unaffected family members. Polymorphic sites were compared to known variants from the NCBI dbSNP database. A total of 27 polymorphisms were identified; 2 were silent, 10 were not translated, and 15 were intronic. Two were homozygous insertions, 2 were deletions, and the remaining 23 were substitutions. Eight polymorphisms were novel. Two of the novel polymorphisms segregated with the affection status for all 24 family members - a 7 base pair deletion in the 5 untranslated region of the SPRY3 gene, and a single base pair intronic polymorphism 22 bases 5 of an alternatively spliced exon of the TMLHE gene. We screened 100 external controls using denaturing high-performance liquid chromatography (DHPLC), and found that 22% exhibited the SPRY3 polymorphism, while 3% contained the TMLHE variant. Further in depth analysis of this contracted interval is underway.

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Association between pain response and an Endothelin Receptor A (EDNRA) polymorphism in children undergoing an IV insertion. *D.L. Schutte[1], C. Kleiber[1], M. Floria-Santos[1], K. Hanrahan[1], J.C. Murray[2], A.M. McCarthy[1].* 1) Col of Nursing, Univ Iowa, Iowa City, IA; 2) Pediatrics, Univ Iowa, Iowa City, IA.

Nearly one half of children report significant pain with IV insertion despite the correct use of topical anesthetics. The purpose of this project, conducted as part of a larger study to test a distraction intervention, was to identify factors that explain variation in topical anesthetic effectiveness used for IV insertion. Children (age 4-10 years), scheduled for an IV insertion, and their parents were recruited. A total of 128 participants met the following inclusion criteria for this subproject: 1) topical anesthetic applied according to recommendations, 2) DNA available, and 3) a completed Oucher scale (self-report measure of pain intensity). An Oucher score of 0-3 was defined as low pain; a score of 4-10 was defined as high pain. Predictor variables included child age and gender, number of previous painful procedures, child temperament (Dimensions of Temperament Scale-Revised), parent anxiety (State-Trait Anxiety Inventory) and alleles in three candidate genes for pain pathways influenced by topical anesthetics [Endothelin-1 (EDN1), Endothelin Receptor A (EDNRA), Endothelin Receptor B (EDNRB)]. All subjects were genotyped for a single SNP in each gene using Taqman methods. Statistically significant differences were found between high and low pain groups on age (high pain group, younger age, p=.0001), child task orientation (higher pain, less orientation, p=0.020), child general activity (higher pain, higher activity, p=0.017), and Endothelin Receptor A genotype (high pain group, TT 67.35%; low pain group, TT 39.47%; p=0.004). A backwards elimination logistic regression procedure identified the EDNRA SNP as the best predictor of the high pain phenotype [Adjusted Odds Ratio=4.13 (1.64-10.440)]. In a follow-up Transmission Disequilibrium Test (TDT), trends of overtransmission of the EDNRA-C allele and undertransmission of the EDNRA-T allele in the low pain response group were noted (p = .085). The identification of genetic and behavioral factors that influence pain response will aid in prescribing the most appropriate pharmacologic and non-pharmacologic interventions.

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The LRRK2 G2019S mutation in Italian Parkinson's Disease. *D. Civitelli, P. Tarantino, G. Nicoletti, I.C. Ciro Candiano, F. Annesi, E.V. De Marco, D. Carrideo, F.E. Rocca, P. Spadafora, G. Annesi.* Inst Neurological Sci, National Research Council, Mangone Cosenza, Italy.

We assessed the frequency of the G2019S mutation of the leucine-rich kinase2 (LRRK2) gene in Italian patients suffering from familial or sporadic Parkinsons disease (PD). This substitution represents the most common causative mutation to date described, and was found in affected members of families with autosomal dominant parkinsonism linked to PARK8 locus (about 5-6%) and in sporadic PD patients (about 2%) in different populations. We screened 38 unrelated familial patients with autosomal dominant parkinsonism and 450 sporadic PD patients. Age at onset was 30-78 years. Moreover, we genotyped 180 healthy subjects from the same geographical area. PCR-RFLP assay of G2019S mutation was performed using Scf1. Among the 38 patients with familial PD, we identified two carriers (5.2%) of G2019S substitution. One of them was heterozygous and the second one, descending from a PD family with consanguineous mating, was homozygous for this mutation. A living affected sibling showed the same rare homozygous genotype. Moreover, we found the heterozygous G2019S mutation in 12 of 450 sporadic PD patients (2.7%). The mutation was absent in controls. Clinical findings were similar between carriers and non-carriers subjects, and included bradykinesia, asymmetric resting tremor and rigidity, and a good response to levodopa. The age at onset ranged 40 to 77 years. The age at onset of homozygous siblings was 47 and 49 years respectively. The LRRK2 gene encodes the dardarin protein. It is the first kinase involved in PD, although its precise function remains unknown. The residue 2019 lies in the highly conserved DYG motif, essential to catalytic function of the protein. The GlySer substitution at this position could result in an impairment of the kinase activity of dardarin. Our results confirm that Gly2019Ser mutation in LRRK2 gene is the most frequent cause of genetic PD in Italians as well as in other different populations. A molecular screening of all 51 exons of the gene could reveal a higher frequency of mutations and increase the weight of dardarin gene in PD.

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A new resistant gene candidate for human narcolepsy identified by a genome-wide association study. *M. Kawashima[1, 2], G. Tamiya[3], H. Hohjoh[2], T. Juji[4], T. Ebisawa[1], Y. Honda[5], H. Inoko[3], K. Tokunaga[2].* 1) Dept Sleep Disorder Research, the Univ. of Tokyo, Tokyo, Japan; 2) Dept Human Genetics, the Univ. of Tokyo, Tokyo, Japan; 3) Department of Moleculer Life Science, Tokai University school of medicine, Kanagawa, Japan; 4) Japanese Red Cross Central Blood Center, Tokyo, Japan; 5) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Human narcolepsy is a sleep disorder that is affected with multiple genetic and environmental factors. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region. To find out associated genetic factors other than HLA, a genome-wide association study using about 23,000 microsatellite markers with pooled DNAs has been performed. The subjects were all Japanese living in the Tokyo area. From the 1st and 2nd screening, >90 microsatellite markers showed statistical significance with Fisher'fs exact test using 2 x 2 tables. Those associations were then assessed by individual typing (case: 228, control: 420), and >30 markers still showed significant differences. Then, a region with one microsatellite marker NA3.4 showing a strong association was subjected to high-density mapping using additional microsatellite markers. In the analysis, a microsatellite marker NA3.L, which is 70 kb distance from NA3.4 showed stronger association than NA3.4 ($p<0.0005$). In succession, a fine mapping using 80 SNPs with the average distance of 5kb was performed. As a result, eight SNPs around NA3.L showed significant associations. In particular, two SNPs located close to the NA3.L showed strong associations (C-4: $p < 0.0005$, C-7: $p < 0.001$). In this region, predicted genes were registered in databases, and we performed expression analyses. In the RT-PCR, we found that a gene tentatively named NLC1-A is expressed in human brain and hypothalamus, as well as spleen, lung, kidney, and skeletal muscle. Moreover, in the reporter gene assay, the expression level was shown to be lower in the constructs with the SNP C-7 and marker NA3.L resistant alleles compared to those with the common alleles. Those observations indicate that the NLC1-A is a resistant gene to human narcolepsy in Japanese.

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Gene expression changes in postmortem brain samples of schizophrenia patients with comparison to matched controls. *H. Chen[1], C.A. Ross[1], M.G. McInnis[2].* 1) Department of Psychiatry & Behavioral Science, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Psychiatry, University of Michigan School of Medicine, Ann Arbor, MI.

Schizophrenia is thought to be due to brain malfunction. Normal brain function at the molecular level requires controlled genetic expression. Although variation in gene expression occurs naturally, there is cumulative evidence of aberrant gene expression in postmortem brains from patients with schizophrenia. Altered expression of genes in the brain that are related to schizophrenia is not fully understood. We performed microarray expression profiling of postmortem brain samples from 15 schizophrenia patients and 15 matched controls using the Affymetrix U133plus2 GeneChip platform. We processed and normalized microarray raw data (CEL files) using the RMA algorithms. Analysis of the normalized data identified 102 genes that showed greater than 40% changes in expression levels in schizophrenia brains compared to controls (nominal t-test p value < 0.05). We further performed TaqMan assay validation of 10 of the 102 microarray significant genes. We observed that expression changes in 7 of the 10 genes tested with TaqMan assays were in agreement with microarray findings, however, there were only three of them with statistical significance ($p < 0.05$). GO database search for functional classification of the 102 significant genes indicates that there are classes of genes involved in biological processes of central nervous system development, synaptic transmission, proteolysis, cell mortality, and small GTPase mediated signal transduction. Our data suggests that modest expression changes are common in most of the significant genes identified with microarray analysis, and a portion of the significant genes may contribute to the malfunction of the schizophrenia brain.

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The aggrecan core protein of precise length is protective of osteoarthritis. *O. Kämäräinen[1], S. Solovieva[2], T. Vehmas[3], K. Luoma[4], P. Leino-Arjas[2], H. Riihimäki[2], L. Ala-Kokko[1], M. Männikkö[1].* 1) Collagen Research Unit, Biocenter and Dept. of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland; 2) Dept. of Epidemiology and Biostatistics, Finnish Institute of Occupational Health, Helsinki, Finland; 3) Dept. of Occupational Medicine, Finnish Institute of Occupational Health, Helsinki, Finland; 4) Dept. of Radiology, Peijas Hospital, Helsinki University, Central Hospital, Vantaa, Finland.

Osteoarthritis (OA), a chronic joint disorder, is characterized by articular cartilage breakdown and joint destruction. It is a major reason for disabilities in the aging population. The imbalance between synthesis and degradation of extra cellular matrix (ECM) molecules is believed to result in the destruction of cartilage leading to pain, stiffness and structural changes in the affected joints. Our goal was to study the contribution of the aggrecan VNTR polymorphism in clinically differing manifestations of hand osteoarthritis. 530 Finnish females representing two academically similar occupations with diverse exposure to mechanical hand load were included. Radiographs of both hands were analysed from all subjects, OA findings were graded and subjects divided into a different categories according to the clinical manifestations. The aggrecan VNTR alleles were analysed by Southern hybridization. Statistical analysis was performed in OA categories comparing joint involvement and pathological findings with the prevalence of the different genotypes. Subjects homozygous for the most common aggrecan VNTR allele, A27 with 27 repeats (n=95), had significantly lower risk for hand OA with OR 0.46 (95% CI 0.27-0.78) for at least mild OA (grade 2 or more), 0.42 (95% CI 0.18-0.96) for severe OA (grade 3 or more), and 0.40 (95 % CI 0.20-0.83) for symmetrical OA. Our results show that carrying two copies of the alleles less than 27 repeats (n=50) exposes to severe forms of hand osteoarthritis (OR 2.45, 95 % CI 1.17-5.12). Carrying two copies of the alleles with more than 27 repeats (n=93) also increased the risk of the disease (OR 1.73, 95 % CI 1.03-2.89). Our findings suggest that a precise number of the repeats is necessary for the optimum function of the aggrecan molecule.

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Identification of candidate gene polymorphisms associated with childhood-onset systemic lupus erythematosus in Mexican population. *R. Velazquez[1], L. Orozco[1], F. Espinoza[3], D. López[3], V. del Castillo[1], V. Baca[2].* 1) Department of Human Genetics, INP, SS, Mexico City; 2) Department of Pediatric Rheumatology, CMN SXXI, IMSS, Mexico City; 3) Department of Immunology, INP, SS, Mexico City.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder that predominantly affects women during their childbearing age. It is estimated that 15-17% of all SLE patients present in children younger than age 16. Susceptibility to SLE has been attributed to complex interactions between genetic and environmental factors. The sibling risk ratio, the disease concordance rate in twins, the identification of several susceptibility loci, and the genetic association to a number of candidate genes support a genetic component of the occurrence of SLE. Initial manifestations of childhood-onset SLE are diverse and often more severe than in adults. Moreover, It has been suggested that genetics may play a greater role in pediatric than in adult SLE. We genotyped 8 single-nucleotide polymorphisms (SNPs) located in genes encoding interleukin-6, interleukin-10, tumor necrosis factor-alpha (TNFA), the low-affinity receptor for the Fc region of IgG (FCGR2A) and the programmed cell death 1 gene (PDCD1), in 170 Mexican pediatric patients with SLE and 200 unrelated, healthy Mexican controls. Genotyping was carried out by the 5 nuclease assay ((TaqMan) or by restriction fragment longitude polymorphism. Genotype frequencies differed significantly between SLE patients and controls for the TNFA -308 and the PD-1.3 polymorphisms (P = 0.004 and P = 0.005 respectively). The A allele frequency of the TNFA -308 polymorphism was more frequent in SLE patients than in controls (8.0% versus 3.0 P = 0.005; OR = 2.56; 95% CI = 1.30-5.04). On the other hand, the frequency of the PD-1.3 A allele was more frequent in SLE patients when compared with healthy controls (5.8% versus 2%, P = 0.005; OR = 2.95; 95% CI = 1.33-6.57). Our results suggest that PD-1.3 and TNFA -308 polymorphisms could play an important role in childhood-onset SLE susceptibility in Mexican population; it also confirms previous associations of these polymorphisms in other populations. This project was partially supported by FOFOI: FP-2003/014.

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Opioid genes and alcohol dependence: whats in and whats not? *X. Xuei[1], L. Flury[1], L. Bierut[2], R. Crowe[3], D. Dick[2], A. Goate[2], J. Nurnberger[1], J. Rice[2], T. Foroud[1], H. Edenberg[1].* 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) Washington Univ Sch Medicine, St. Louis, MO; 3) Univ of Iowa, Iowa City, IW.

Opioid receptors and their endogenous peptide ligands play an important role in neurotransmission and neuromodulation, and in the rewarding and reinforcing properties of drugs such as cocaine, heroin, and alcohol. There are three known classes of opioid receptors: mu, delta, and kappa. The interactions of the opioid receptors with their peptide ligands usually lead to the activation of neurotransmission. There have been reports that variations in OPRM1 are associated with alcoholism, but data were not consistent, some were positive while others were negative. To examine whether the opioid system is associated with the risk for alcoholism, we performed extensive SNP genotyping on OPRM1 (18 SNPs), OPRD1 (16 SNPs), OPRK1 (13 SNPs), OPRL1 (8 SNPs), PDYN (18 SNPs) and PENK (5 SNPs) genes in the large family-based Collaborative Study on the Genetics of Alcoholism. Analyses were done with the Pedigree Disequilibrium Test (Martin, 2000) using 1860 Caucasian American individuals from 218 multiplex alcohol families. The data did not support the association of OPRM1, OPRD1, and OPRL1 genes with alcohol dependence. However, 7 consecutive SNPs in intron 2 of OPRK1 were significantly associated with alcohol dependence (p 0.05). The kappa-opioid receptor has been reported to produce aversive states, thus may prevent the development of reinforcement. Eleven SNPs in the PDYN gene also were associated with alcohol dependence. Haplotype analysis using a three-SNP sliding window further confirmed the association of both genes, with p-values as low as 0.00009. Therefore, we conclude that the variations of OPRK1, encoding the kappa-opioid receptor, and PDYN, encoding its ligand, are strongly associated with the risk for alcoholism. The finding that both the kappa-opioid receptor and its ligand are associated with alcohol dependence makes biological sense.

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Update on identifying drug targets associated with human disease. *P. St Jean, L. Li, D. Montgomery, R. Newton, D. Yarnall, M. Sumner, J. Stuart, L. Middleton, D. Burns, E. Lai, S. Chissoe, A. Roses.* Genetics Research, GlaxoSmithKline, Res Triangle Park, NC, Harlow and Stevenage UK, Upper Merion and Providence, PA.

Over the past decade the pharmaceutical industry has seen decreased productivity in drug discovery. The GSK HiTDIP program (High Throughput human-Disease specific target Program) was initiated to deliver drug targets genetically associated with human disease (a target is a protein thought to play a key role in disease pathophysiology and whose activity may be modified by drug therapy). The premise of this program is that identification of disease associated drug targets will reduce attrition in the drug discovery pipeline. Over the next several years, this premise will be borne out by a larger proportion of successful Phase IIA efficacy studies (Phase II is usually considered the stage at which the drug is evaluated for effectiveness in patients). The value of HiTDIP in enhancing drug discovery can then be evaluated against historical methods of target selection. The HiTDIP program, in conjunction with physician collaborators, has recruited large, well phenotyped case/control collections for many different common human diseases in respiratory, inflammatory, musculoskeletal, cardiovascular, metabolic, neurological and psychiatric disease areas. These collections of approximately 1000 cases and 1000 controls have been genotyped for SNPs in ~2000 target genes (such as 7TMs, kinases and ion channels) and then tested for association to disease status. The analysis design uses an initial test set and a replication set. To date the HiTDIP program has delivered results for 12 initial test screens and 4 replication screens. Some well-known associations have been identified such as the HLA region in Rheumatoid Arthritis and APOE in Alzheimers Disease. This poster will give an overview of the analytical methods applied and highlight interesting features of results from the some of the completed studies.

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HLA risk haplotypes segregating in a multiplex family affected with systemic lupus erythomatosus (SLE). *P.R. Pacheco[1,2], M. Pereira[1], L. Mauricio[1], M. Baptista[1], A. Quental[1], C. Paiva[1], L. Mota-Vieira[1,2].* 1) Hospital do Divino Espirito Santo, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

SLE is a chronic autoimmune disorder, caused by the production of pathogenic autoantibodies with specificity for nuclear and other tissue antigens. Although the genetic basis for SLE susceptibility is not known, recent studies in several populations support a role for specific HLA alleles and extended haplotypes, spanning the class II region, as genetic risk factors for SLE expression. Here, we report the identification of one extended and one class II haplotypes segregating in an Azorean family from Sao Miguel island, in whom the propositus - a women (51y) with classical SLE - has all three sons affected with different expressions of the disease: the 29y has a asymptomatic SLE, the 24y has a lupus nephritis, and the youngest (20y) had an episodic exanthema. All four have or had a positive antinuclear antibodies. Blood samples were collected from all patients, as well as from two non-affected family members: the husbands and mothers propositus. The HLA genotyping for class I (A, B and Cw) and class II (DRB1, DQA1 and DQB1) was carried out using PCR-SSP from Olerup SSP. Segregation analysis revealed that the haplotype (A*01-B*08-DRB1*03-DQB1*02) strongly associated with SLE in Caucasians is present in the propositus and in the two oldest sons, but absent in the youngest one. Moreover, the three boys also inherited, from his father, a class II risk haplotype associated with SLE (DRB1*15-DQB1*06). These alleles have relatively low frequencies in Sao Miguel population (0.075% and 0.198%, respectively). Considering these results, we are now genotyping all class II alleles, with high resolution (4 digits), because it is known that the DRB1*1501-DQA1*0101 has a multiplying effect on the SLE expression and is implicated in lupus nephritis. Moreover, an extended familial study is planned with a particular emphasis for other propositus relatives. This type of familial study is very useful for the patients clinical follow-up, and essential for understanding the susceptibility of autoimmune diseases.

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Association of *FOXP3* gene variants with Systemic Lupus Erythematosus (SLE). *M. Barreto[1], R. Ferreira[1], L. Lourenço[1], C. Fesel[1], J. Demengeot[1], R. Andreia[2], J.F. Viana[2], C. Vasconcelos[2], B. Martins[3,4], L. Mota-Vieira[5], C. Ferreira[2,6], A.M. Vicente[1,7].* 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Associação dos Doentes com Lupus, Lisboa, Portugal; 3) Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal; 4) Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal; 5) Hospital do Divino Espírito Santo, Ponta Delgada, Azores, Portugal; 6) Clínica Universitária de Medicina II, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; 7) Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal.

FOXP3 is a member of forkhead/winged-helix proteins, which is involved in the regulation of T-cell activation, and therefore essential for normal immune homeostasis. It is specifically expressed by $CD4^+CD25^+CD45RO^+$ regulatory T cells (Treg), which are crucial in the prevention of autoimmune disorders due to their ability to suppress deleterious immune responses against self-antigens. To examine the involvement of the *FOXP3* gene in SLE susceptibility, we tested the association of the disease with two polymorphisms in this gene: a microsatellite marker on intron 0 in the enhancer/promoter region, which has been previously reported to regulate FOXP3 mRNA levels, and another microsatellite in intron 5 in a population consisting in 157 patients and 98 healthy individuals. The polymorphism in intron 0 showed a significant association with SLE (P=0.036). Given the putative role of this polymorphism in FOXP3 expression, we compared FOXP3 mRNA expression levels in patients with healthy controls. SLE patients showed higher levels of FOXP3 mRNA than healthy individuals, although not reaching statistical significance, possibly due to the small number of tested individuals. No association was found between *FOXP3* polymorphisms and its expression levels. We therefore screened the *FOXP3* gene for polymorphisms in the promoter/enhancer region that could influence the observed difference in FOXP3 expression. Given that no other variants were found in this region, we believe that variation in the intron 0 microsatellite polymorphism may influence the binding of regulatory proteins and thus modulate susceptibility to SLE.

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TNF polymorphisms and occupational respiratory disease in a rural cohort. *R.E. Slager[1], J.L. Meza[2], S.E. Puumala[2], D.J. Romberger[1], S. von Essen[1].* 1) Dept Internal Medicine, Univ. of Nebraska Medical Center, Omaha, NE; 2) Preventive & Societal Medicine, Univ. of Nebraska Medical Center, Omaha, NE.

The genetic contribution of tumor necrosis factor (TNF) polymorphisms to asthma and other complex airway diseases is controversial. The functional significance of several well-characterized single nucleotide polymorphisms (SNPs) within the adjacent genes for TNF- (*TNFA*) and lymphotoxin and TNF- (*LTA*) is also not well understood. Few studies have focused on the association between TNF SNPs and respiratory disease in adult farmers of midwestern American. These individuals have specific respiratory challenges, including exposure to potentially high levels of endotoxin and particulate matter from inhalation of organic dusts in the agricultural setting. We now report genotyping results for two TNF polymorphisms, the *TNFA* -308G>A promoter polymorphism (dbSNP rs1800629) and an *Nco*I restriction fragment length polymorphism (RFLP) within the first intron of *LTA* (dbSNP rs909253), as well as the demographic profile of our rural cohort (n=199), composed primarily of farmers from Nebraska. Health and occupational data was collected from questionnaires, lung function was measured by spirometry, and genomic DNA was isolated from whole blood. The median age of our cohort was 57 and 73% of participants reported central European heritage. The median FEV_1 % predicted was 94.0 and the median FVC % predicted was 99.0. Smoking history was also taken into account, as 9% of individuals were current smokers and 25% were former smokers (median pack years 15). Genotyping was performed by PCR and RFLP analysis and confirmed by sequencing. The allele frequency in our population at the *TNFA* -308 locus was: 0.82 allele 1 / 0.18 allele 2, which is consistent with published frequencies in Caucasian populations. The allele frequency of the *LTA* SNP was: 0.36 allele 1 / 0.64 allele 2. Phenotypic information will be used to determine if there is a correlation between specific TNF alleles and respiratory function or disease as well as to quantitate environmental exposure in order to define gene / environment interactions.

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Interaction of CFH T1277C polymorphism and cigarette smoking in age-related macular degeneration. *W.K. Scott[1], S. Schmidt[1], M.A. Hauser[1], P. Gallins[1], S. Kwan[1], L.M. Olson[2], N. Schnetz-Boutaud[2], K.L. Spencer[2], J.R. Gilbert[1], A. Agarwal[2], E.A. Postel[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville TN.

Age-related macular degeneration (AMD) is a complex disease influenced by genetic and environmental risk factors. A strong association between the CFH T1277C polymorphism and AMD was reported in several independent samples, making it the most widely replicated genetic risk factor for AMD. Cigarette smoking is the most consistently implicated environmental risk factor for AMD. Both CFH and smoking influence complement activation. Thus, we examined potential gene-environment interaction between these two strong risk factors for AMD. 438 people with AMD and 142 unrelated controls were ascertained from two university eye clinics. Standard criteria were used to rate severity of macular disease (grades 1-5) based on examination of fundus photographs. Individuals were classified as ever or never smokers based on self-reported smoking of 100 cigarettes. T1277C genotypes were determined by sequencing of the polymorphic site. Logistic regression analyzed the joint effect of smoking and genotype, controlling for age and sex and including two-way interaction terms for smoking and genotype. Borderline significant interactions between smoking and the TC genotype were detected overall (p=0.09) and when comparing 276 grade 5 (neovascular) AMD cases to 109 grade 1 controls (p=0.09). Compared to a referent of never smokers with the TT genotype, grade 5 cases were more likely than controls to carry the CC genotype regardless of smoking history (OR=15.3, 95% CI: 3.7-63.8 in never smokers; OR=27.8, 95% CI: 7.4-104.9 in smokers). However, grade 5 cases were only more likely than controls to carry the TC genotype among smokers (OR=10.6, 95% CI: 3.2-34.7); in non-smokers, the TC genotype was not associated with AMD (OR=1.7, 95% CI: 0.5-5.1). A similar, but less significant, pattern was observed when comparing all cases to all controls. These results suggest that cigarette smoking might modify the effect of the TC genotype on risk of AMD.

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Parent-of-origin effects in Attention Deficit Hyperactivity Disorder (ADHD). *L. Goos, R. Schachar.* Brain & Behaviour Res Program, Hosp Sick Children, Toronto, ON, Canada.

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurological disorders of childhood, affecting approximately 3-7 percent of all school age children. Generally characterized by age-inappropriate and impairing levels of inattention, hyperactivity and impulsiveness, many children also display varying degrees of intellectual impairment, cognitive deficits, and comorbid psychopathologies. Despite no consistent sex differences in core symptoms, severity or level of impairment, ADHD is 2-9 times more prevalent in boys than in girls. According to threshold models of ADHD etiology, this disparity is due to sex differences in the burden of genetic and environmental risk factors required to produce clinically significant symptoms. Thus, ADHD in girls represents the accumulation of a greater number of risk factors; where these risks are genetic in origin, females with ADHD may also transmit a greater number of risk factors to their offspring. In this study, the influence of sex on the transmission and manifestation of ADHD was investigated. The behavioural, intellectual and emotional characteristics of girls and boys diagnosed with ADHD were evaluated for parent-of-origin effects. Maternal history of ADHD was significantly associated with greater behavioural symptoms in children of both sexes. Paternal history of ADHD interacted with proband sex on intellectual and emotional measures, resulting in greater impairment in girls relative to boys in these domains. Interpretations of these data in terms of environmental and genetic factors, including epigenetic and sex-linked hypotheses, are explored.

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Identification of Novel Asthma Susceptibility Loci on Chromosome 12q. *B.A. Raby[1], R. Lazarus[1], J. Su[2], K. Schneiter[2], C. Lange[2], E.K. Silverman[1], S.T. Weiss[1].* 1) Channing Laboratory, Brigham & Women's Hospital, Boston, MA; 2) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Chromosome 12q is among the regions most commonly linked to asthma in both genome-wide and regional linkage studies, yet the gene(s) responsible for these linkages have yet to be identified. We have previously demonstrated evidence for linkage with asthma to this region in families ascertained through the Childhood Asthma Management Program (CAMP). We subsequently performed high-resolution linkage disequilibrium (LD) mapping across this region using 1472 LD-tagging SNPs in 395 Caucasian CAMP families and identified 31 regions with significant evidence ($p<0.05$) of association with asthma. We now present results of follow-up SNP genotyping in these associated regions at substantially higher resolution. 535 markers were genotyped in these 31 associated regions to achieve a target SNP density of 5kb. Of the 31 regions, only 8 demonstrated persistent evidence of association in the second round of genotyping, with 2 regions demonstrating very strong evidence of both single-SNP and haplotype transmission distortion. Region 1 spans 176kb and included 26 SNPs demonstrating significant transmission distortion (transmission:non-transmission ratio 1.6:1, $p_{min}=0.0006$). Haplotype analysis revealed transmission distortion of one common haplotype at 18% frequency (global haplotype test $p=0.002$; haplotype-specific $p_{min}=0.0005$). Region 2 spans 37kb and includes 8 SNPs demonstrating significant transmission distortion (T:U ratio 1.4:1, $p_{min}=0.0002$). SNPs in this region form only two common haplotypes of 28% and 69% frequency. Survival analysis of time-to-symptom-onset revealed that both regions were significantly associated with earlier onset of asthma symptoms ($p_{min}=0.008$ and 0.002, respectively). There are five known genes mapping to these associated regions, none of which has previously been implicated in asthma, suggesting they may provide new insights into the pathobiology of this disease.

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Haplotype Analysis of Complement Factor H and the CFH-like Genes and Risk for Age-related Macular Degeneration. *K.M. Spencer[1], M.A. Hauser[2], S. Schmidt[2], W.K. Scott[2], L.M. Olson[1], P. Gallins[2], S.Y. Kwan[2], M. Noureddine[2], J.R. Gilbert[2], N. Schnetz-Boutaud[1], A. Agarwal[3], E.A. Postel[4], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Center for Human Genetics and Department of Medicine, Duke University Medical Center, Durham, NC; 3) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; 4) Duke University Eye Center and Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly. Estimates suggest that over 50 million people worldwide are affected with this disorder, including 15 million in the United States. Recently, Haines et. al (2005) and other groups reported a strong association between the Y402H variant in the complement factor H gene (CFH) and risk for AMD. The CFH gene and 5 related CFH-like genes all lie within the Regulator of Complement Activation (RCA) gene cluster on chromosome 1q32. Because these genes may play a role in control of the complement system and epistatic interactions may exist between polymorphisms in these genes, the CFH-like genes were also sequenced in a subset of our AMD dataset. Fifteen known SNPs were observed and 8 novel SNPs were discovered. Strikingly, in the 48 people sequenced we observed a marked reduction in variation within the CFH and CFH-like genes in cases compared to controls ($p < 0.0001$), which further implicates the Y402H polymorphism in AMD risk. This may also indicate that the AMD risk haplotype is of recent evolutionary origin. After genotyping 14 coding SNPs observed in our sequenced samples in our full AMD case-control dataset of 465 cases and 170 controls, the reduction in variation was confirmed ($p < 0.0001$). Evidence for a protective haplotype spanning the CFH gene was also obtained ($p < 0.01$). This haplotype is significantly underrepresented in cases versus controls, after taking into account the overrepresentation of the Y402H risk haplotype in the cases.

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Identifying schizophrenia-associated non-coding variants in the CAPON gene. *N.S. Wratten[1], S.E. Bruse[1], M.A. Azaro[1], J. Simone[1], J.E. Hayter[1], L.M. Brzustowicz[1,2].* 1) Dept of Genetics, Rutgers University, Piscataway, NJ; 2) Dept of Psychiatry, UMDNJ-NJMS, Newark, NJ.

We have previously shown linkage between chromosome 1q23 and schizophrenia and detected significant LD between schizophrenia and markers that fall within the CAPON gene in a set of families of European descent. A mutation screen of these samples failed to identify any associated coding SNPs. An independent study in a Han Chinese sample found a different pattern of significant association between markers in this gene and schizophrenia[3]. CAPON spans 300 kb and a number of statistical analyses have indicated more than one region of the gene may be associated with the disease. Heterogeneity in disease associated markers is often seen in complex traits and could be explained by mutations in independent regulatory modules. Regulatory sequences are not constrained by position relative to coding sequences so it is important to examine markers throughout the gene, including 5 and 3 regions. Given the large size of CAPON, it was necessary to use an efficient tagSNP approach to survey the entire gene with a minimal set of SNPs. Since established tagSNP approaches have certain limitations, we combined two methods; the Perlegen database that groups SNPs into LD bins based on r^2 (http://genome.perlegen.com/) and the Applera SNP browser software that chooses SNPs based on LDUs created with the measure (http://www.applera.com/). We required that a SNP was chosen from each bin and at a density of 0.33 LDUs. Luminex assays were then developed for the resulting panel of 53 SNPs (including the 15 SNPs previously reported) using custom software to allow the multiplexing of 10 to 15 SNPs per PCR reaction, substantially reducing the cost and the time to genotype samples. Associated SNPs give an approximate location for potential regulatory sequences, with nearby conserved sequences then tested for regulatory function in Hela and neuronal cell lines. This analysis pipeline allows for the efficient and systematic evaluation of even a large candidate gene for potential causative regulatory mutations. [3]Zheng *et. al.*, Biochem Biophys Res Commun. 2005, 328(4):809-15.

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Bipolar 1 disorder and schizophrenia: A 440-SNP screen of 64 candidate genes among familial Ashkenazi Jewish case-parent trios. *A.E. Pulver[1,2], M.D. Fallin[2], V.K. Lasseter[1], N. Cheng[1], J.A. McGrath[1], K.Y. Liang[3], G. Nestadt[1], D. Valle[4,5], D. Avramopoulos[1], P.S. Wolyniec[1].* 1) Dept Psychiatry, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Department of Epidemiology, The Johns Hopkins Bloomberg School of Public Health; 3) Department of Biostatistics, The Johns Hopkins Bloomberg School of Public Health; 4) McKusick-Nathans Institute, The Johns Hopkins School of Medicine; 5) Howard Hughes Institute, the Johns Hopkins School of Medicine.

Schizophrenia (SZ), schizoaffective (SZA), and bipolar 1 disorders (BP1) are common heritable psychiatric disorders, for which familial co-aggregation as well as epidemiological and genetic evidence suggest genetic heterogeneity and overlapping etiologies. In an attempt to reduce genetic heterogeneity, we have ascertained families of Ashkenazi Jewish descent. As an initial effort, a SNP screen of 64 candidate genes was genotyped with an average 6.9 SNPs per gene and an average of 1 SNP every 11.9 Kb in 274 SZ and SZA and 323 BP1 Ashkenazi case-parent trios. Using single SNP and haplotype-based transmission disequilibrium tests, we ranked genes based on p<.01. Six genes (DAO, GRM3, GRM4, GRIN2B, IL2RB, and TUBA8) were associated with susceptibility for BP1 and six genes (RGS4, SCA1, GRM4, DPYSL2, NOS1, and GRID1) were associated with SZ/SZA. In addition, several genes showed overlapping suggestive evidence of association in both datasets (DPYSL2, DTNBP1, G30/G72, GRID1, and GRM4). To further reduce heterogeneity, we have now completed a stratified analysis, i.e., we examined the subset of familial trios in the SZ/SZA and BP1 samples using the same methods as above. For the 126 familial BP1 trios, GRIN2B and GRID1 are associated at the p<.01 level. For the 112 familial SZ/SZA trios, 6 genes are associated at the p<.01 level: ADRA1A, SCA1, KIF13A, NRG3, HTR7, HTR2A, and PRODH. Despite the decreased sample size, stratifying the proband groups by family history of disease supports previously found associations and potentially identifies additional genes (ADRA1A, KIF13A, NRG3, HTR7, HTR2A, and PRODH) that may play a role in susceptibility to subgroups of the case populations.

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Analysis of chromosome 10 LOD-1 linkage region to identify genes associated with late onset Alzheimer's Disease. *A.R. Morgan[1], L. Jehu[1], D. Turic[1], G. Hamilton[2], L. Busby[2], J. Powell[2], S. Lovestone[2], M. Owen[1], J. Williams[1].* 1) Cardiff University Dept of Psychological Medicine, University Hospital of Wales, Heath Park, Cardiff CF14 4XN; 2) Departments of Old Age Psychiatry and Neuroscience, Institute of Psychiatry De Crespigny Park London SE5 8AF, UK.

In our genome scan for linkage with LOAD we observed significant evidence of linkage to chromosome 10 spanning approx 44 cm from D10S1426 (59cM) to D10S2327 (103cM) (Myers et al., 2002). In this current study we have focussed on the Max LOD -1.0 region, defined as the area encompassed by all markers producing LOD scores between approx 2.83 and 3.83, flanked by markers D10S1220 (70cM) and D10S1670 (86cM). All known and predicted genes between these markers were downloaded from the Ensembl database, and SNPs were identified within the genes using db SNP.

Using the SNPs, with an average SNP density 5.5kb (862 SNPs in 80 genes), we tested for association with LOAD. SNPs were typed in pooled samples of 4 case and 4 control DNA pools, totalling 366 case subjects and 366 control individuals. We obtained results for 494 SNPs. 23 SNPs (in 13 genes) gave significant results after correction with het ratio: VR22 (5 SNPs), LCX (1 SNP), ANK3 (3 SNPs), PLEAKHA1 (1 SNP), EGR2 (1 SNP) JMJDIC (1 SNP), OGDHL (1 SNP), TIMM23 (1 SNP), TMEM23 (3 SNPs), ACF (1 SNP), CGK1 (1 SNP), Em: AC074327. 1 (1 SNP), PCDH15 (3 SNPs)

These 23 positive SNPs now require genotyping in additional pooled DNA to further support our significant associations.

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Genetic variants in *IRF6* are strongly associated with orofacial clefts in a Norwegian Population. *F. Rahimov[1], A. Jugessur[1,3], AJ. Wilcox[2], RT. Lie[3], HK. Gjessing[4], JC. Murray[1].* 1) University of Iowa, Iowa City, IA; 2) National Institute of Environmental Health Sciences, Durham, NC; 3) University of Bergen, Norway; 4) Norwegian Institute of Public Health, Norway.

Recently, mutations in the gene encoding interferon regulatory factor 6 (*IRF6*) were shown to underlie Van der Woude syndrome (VWS), in which lower lip pits and occasional hypodontia are the only additional features distinguishing the syndrome from isolated clefts. To date, two studies have reported strong associations of *IRF6* variants with CL/P. In a population-based case-control study of orofacial clefts in Norway (1996-2001), DNA was available from babies and their parents, and occasionally from additional siblings. The study had a high participation rate of 88% in the case group (n=574) and 76% in the control group (n=763). Of these, 187 were complete CPO triads, 135 were CLO, 228 were CLP, and 372 were control triads. Five SNPs (rs669694, rs4844880, rs126280, rs2013162, and rs17015215) at the *IRF6* locus were selected based on preliminary results showing an association with isolated CL/P in a Danish and Filipino population. All analyses were performed using HAPLIN (see http://www.uib.no/smis/gjessing/genetics/software/haplin), which enables estimation of the effects of a single or double dose of an allele or haplotype. A major strength of HAPLIN is that both fetal and maternal effects can be assessed separately, which is especially relevant when studying birth defects. Whereas no significant effects, either fetal or maternal, were observed in CPO, the cleft lip categories, however, showed significant distortions in the transmission of alleles for several of the markers. Haplotype-based analyses revealed a high-risk haplotype C-T-A-C-C (SNP order as above) in the CLO subgroup of clefts. The fetal haplotype relative risk was 2.6 (95%CI: 1.4-4.7; $p < 0.01$) with a single-dose of this haplotype and 5.2 (1.3-20) (p=0.02) with two doses. Combining the cleft lip subgroups does not appreciably alter these estimates (single-dose, 1.8 (1.2-2.7), $p < 0.01$; double-dose, 2.8 (1.1-6.8), p=0.03). In conclusion, these findings confirm the role of *IRF6* variants in clefting of the lip in the Norwegian population.

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Association between OPRM1 Gene Variants and Substance Dependence in European Americans. *H. Zhang[1,2], H.R. Kranzler[3], X. Luo[1,2], B.Z. Yang[1,2], J. Lappalainen[1,2], J. Gelernter[1,2].* 1) Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT; 2) VA Connecticut Healthcare System, West Haven, CT; 3) Dept. of Psychiatry, University of Connecticut School of Medicine, Farmington, CT.

Previous association studies of the -opioid receptor gene (*OPRM1*) in substance dependence have focused on a few gene variants without full coverage of the gene, and findings are inconsistent. In this study, we examined a more extensive set of *OPRM1* single nucleotide polymorphisms (SNPs) in a sample of 406 European Americans (EAs) affected with substance dependence (337 with alcohol dependence and 204 with cocaine and/or opiate dependence) and 328 EA healthy controls. 13 SNPs spanning *OPRM1* were selected and genotyped. The SNPs were found to be located in two haplotype blocks. The genotype distributions of all SNPs were in Hardy-Weinberg equilibrium (HWE) in controls; but in cases, 5 SNPs in Block I and 3 SNPs in Block II showed genotype distribution deviation from HWE. Significant differences were found between cases and controls in both allele and genotype frequencies for 4 SNPs in Block I and 2 SNPs in Block II. Association of one SNP in Block I with drug dependence and two SNPs in Block II with alcohol dependence remained significant after correcting for multiple testing. Logistic regression analyses also indicated that one SNP in Block I and another SNP in Block II were associated with drug and alcohol dependence, respectively, when the sex and age of subjects, two potential confounding factors, were considered. Haplotype case-control studies showed that the frequency distribution of haplotypes (constructed by either the 7 SNPs in Block I or the 6 SNPs in Block II) was significantly different between cases and controls (haplotypes in Block I: $P = 0.020$ and 0.030 for alcohol and drug dependence, respectively; haplotypes in Block II: $P = 0.010$ for both alcohol and drug dependence). Structured association analyses using 37 ancestry-informative markers (AIMs) excluded the possibility that the positive findings resulted from population stratification and admixture effects. These results strongly support that *OPRM1* variants are associated with substance (alcohol and/or drug) dependence.

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Genome-wide scan for Adiposity-related Phenotypes in Adult Samoans. *F. Dai[1], E.D. Keighley[2], G. Sun[3], D. Smelser[3], S. Viali[4], J. Tuitele[5], L. Jin[3], R. Deka[3], D.E. Weeks[6,1], S.T. McGarvey[2].* 1) Dept of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) International Health Institute, Brown University, Providence, RI; 3) Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH; 4) Tupua Tamasese Meaole Hospital, Apia, Samoa; 5) Tafuna Family Health Center, Pago Pago, American Samoa; 6) Dept of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

As part of a genetic epidemiology study of adiposity in Samoa and American Samoa, we conducted a genome scan to detect linkage with adiposity QTLs among 677 adults from large pedigrees in American Samoa. The probands and family members were unselected for obesity and were recruited from all regions of the Samoan archipelago. Adiposity was assessed by BMI, abdominal circumference (ABDCIR), percent body fat (%BF), measured by bioelectrical impedance, and fasting serum leptin and adiponectin. The distribution of all phenotypes was transformed to normality, and adjusted for the influence of age and sex. The self-reported pedigree relationships were checked and corrected after genotyping. Multipoint LOD scores were calculated using variance components methods and SOLAR/LOKI software. Adiposity QTLs with LOD2.0 were detected for the following regions: (A) 16q21-23: LOD = 2.44 for BMI, LOD = 2.25 for %BF (and LOD = 1.98 for ABDCIR, LOD = 1.53 for leptin); (B) 13q33: LOD = 2.25 for adiponectin; (C) 4p15: LOD = 2.07 for BMI. These preliminary linkage analyses indicate potential pleiotropy at 16q21-23. Our work is supported by NIH grant R01-DK59642 (S.M. PI).

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Fine mapping and candidate gene screening of 3q25-27 in Finnish autism families. *K. Rehnström[1,2], T. Ylisaukko-oja[1,2], R. Vanhala[3], R. Alen[4], R. Riikonen[5], E. Kempas[2], H. Komu[1], L. Peltonen[2], I. Järvelä[1].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 4) Department of Child Neurology, Central Hospital of Central Finland, Jyväskylä, Finland; 5) Department of Child Neurology, Children's Hospital, University of Kuopio, Kuopio, Finland.

Linkage to 3q25-27 has previously been reported in a Finnish genome-wide screen for autism spectrum disorders. We have now fine-mapped this region by linkage and association methods using a dense set of microsatellite markers in an extended set of families (n=100) including the families from the original genome-wide screen. Linkage to the 3q25-27 region was maintained in this extended patient sample (D3S3037 resulting in a two-point LOD score exceeding 3) and suggestive association was observed at D3S3037 and at a polymorphic dinucleotide repeat intragenic to MCF2L2, a rho-family guanine exchange factor (PSEUDOMARKER p=0.018 and p=0.049, respectively). Possible candidate genes involved in neuronal development and function were identified in silico in the region. Four candidate gene regions containing six genes, neuroligin 1 (NLGN1), ephrin receptor EphB3 (EPHB3), fragile X mental retardation-related protein 1 (FXR1), and three serotonin receptor 3 subunits (HTR3C, HTR3D and HTR3E) were chosen for further analysis. Coding regions of these genes were screened for mutations by sequencing in a subset of patients. Sequence analysis of NLGN1 did not reveal any functional mutations, and only one SNP resulted in suggestive association (FBAT p=0.002). Therefore we conclude that our results do not provide strong evidence for the involvement of NLGN1 in the etiology of autism in the Finnish material. For the other candidate genes, a dense set of SNP markers will be genotyped in all families in order to detect susceptibility variants in non-coding regions. Family based association and haplotype association tests will be performed to assess their importance in autism spectrum disorders.

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Genetic variation in 5-hydroxy-tryptamine receptor (5HTR2A) is associated with obesity in the NHLBI Family Heart Study. *I.B. Borecki[1], Y. Jiang[2], S. Williamson[2], K.E. North[3], D. Ma[1], M.A. Province[1], R.H. Myers[2].* 1) Div Biostatistics, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Neurology, Boston Univ Sch Medicine, Boston, MA; 3) Dept Epidemiology, Univ NC, Chapel Hill, NC.

A genome-wide linkage scan of BMI in Caucasian pedigrees from the Family Heart Study (Feitosa et al. 2002) revealed evidence of QTLs on chromosomes 7q31.3 (lod=4.9) and 13q14 (lod=3.2); Black families were not genotyped in that project phase. To follow up on these signals, 82 families (N=518) and 130 families (N=1104) with the largest contribution to the respective lod scores on 7 and 13 were studied. The focus of the present study is to test the potential role of the 5HTR2A locus, which maps within the peak on 13 at ~45,250 kb. Serotonin (5-HT) is a key mediator in the control of satiety and is probably involved with weight regulation (Leibowitz 1990), and a polymorphism in the 5HTR2A gene has been shown to be associated with dietary energy and alcohol intake in obese subjects (Aubert et al. 2000). A preliminary study was carried out in the chromosome 7 set of families using a particular SNP in the 5HTR2A gene (dbSNP ID: rs6313) because it appears to be a functional mutation - it introduces a missense mutation (Arg(CGG)39Trp(TGG)) in the second exon of one spliced mRNA form, and it also introduces a silent mutation in other transcription forms. A significant association with obesity (BMI > ~ 30 kg/m2) was found (P=0.007) using a family-based TDT implemented in TRANSMIT. We have now typed 11 SNPs across the gene in the independent set of chromosome 13 families; all but one (rs1923885; p=0.04) were in Hardy-Weinberg equilibrium. We replicated an association with 5HTR2A, although for a different SNP (rs2770296, p=0.04) and a 3-SNP haplotype including its flanking markers (p=0.01). While the two associated SNPs are in linkage disequilibrium (r^2~0.4), these results make room for the possibility that there may be heterogeneity among the haplotypes harboring functional variants. Therefore, while we have replicated evidence for the association of variants in 5HTR2A with obesity, further work is necessary to understand precisely which variants are functional.

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Association Study of Trace Amine Receptors in Bipolar Disorder. *C. Liu, J. Shi, H. Zou, J.A. Badner, S.L. Christian, E.S. Gershon.* Dept Psychiatry, Knapp Res Ctr, Univ Chicago, Chicago, IL.

Trace amines including tyramine, beta-phenylethylamine, tryptamine, and octopamine are biogenic amines. Some of them have been recognized as neurotransmitters in invertebrates. A family of trace amine receptors has been discovered in a small region at human 6q23.2. 6q24 has been reported to link to both SZ and BD. Although their biological functions are mostly unclear, one of the trace amine receptors (TRAR4, named as TAAR6 by Lindemann et al. 2005) was reported to be associated with schizophrenia (SZ). Twenty-five SNPs were selected for association test in bipolar disorder (BD) samples from the ~122 Kb regions where all nine known human trace amine receptors located. 557 individuals from 177 Caucasian pedigrees with BD were studied. Transmission/disequilibrium test (TDT) and haplotype analysis were performed. None of the associated SNPs in SZ -- rs8192625, rs4305745, rs6903874 and rs6937506 -- showed association with BD. Two distal markers (rs9389008 and rs9321360) showed nominally significant association. rs9389008 is close to TAAR4; rs9321360 is 8 Kb upstream to TAAR1. None of the results were significant after correcting for multiple testing using permutations. Haplotype block and LD map data indicate that the selected markers provided adequate coverage for this genomic region. Thus, trace amine system may not play a major role in bipolar disorder on 6q.

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***FOXO3A* is associated with human longevity.** *L. Pawlikowska[1], D. Lind[1], E. Lovins[1], T.J. Nguyen[1], J. Chen[1], C. Chu[1], S. Huntsman[1], W. Browner[2], W.C. Hsueh[1], P.Y. Kwok[1], E. Ziv[1].* 1) Ctr for Human Genetics & Dept of Medicine, University of California, San Francisco, CA; 2) California Pacific Medical Center Research Institute, San Francisco, CA.

Genes in the insulin/IGF1 signaling pathways affect lifespan in model organisms. In *C. elegans*, mutations in *daf-2*, the homologue of insulin/IGF1 receptors, lead to increased resistance to oxidative stress and longer lifespan. These mechanisms appear conserved through evolution: mice with IGF1 receptor haploinsufficiency have increased lifespan. In *C. elegans*, the effect on lifespan requires the transcriptional factor DAF-16. FOXO3A, a mammalian homologue of DAF-16, increases oxidative stress resistance. We hypothesized that common polymorphisms in *FOXO3A* may be associated with variation in human lifespan. To identify *FOXO3A* single nucleotide polymorphisms (SNPs), we sequenced 6.7 kb of genomic sequence including exons, exon-intron boundaries and 1kb of promoter. Haplotype tag SNPs (htSNPs) were selected by eliminating redundant SNPs (R^2=1.0). We genotyped six htSNPs in 896 Caucasian women from the Study of Osteoporotic Fractures, selected for near-normal (n=603, lifespan79y, mean=75.72.6y) vs. extreme long lifespan (n=293, lifespan92y, mean=95.32.1y). We tested the association of genotype with lifespan by comparing individual SNP allele frequencies and computationally predicted haplotype frequencies between long-lived individuals and controls. All p-values are unadjusted. Five of the six *FOXO3A* htSNPs were associated with lifespan ($p<0.05$). We observed the strongest effect for an intronic SNP (minor allele frequency 0.19 vs. 0.13, p=0.0015) and the Ala53 synonymous SNP (0.17 vs. 0.12, p=0.004). Global haplotype distribution differed between long-lived women and controls (p=0.01). One haplotype was overrepresented among long-lived cases (frequency 0.17 vs. 0.12, p=0.003); the most common haplotype was underrepresented (0.64 vs. 0.70, p=0.02). In conclusion, genetic variants in *FOXO3A* may be associated with longer lifespan in Caucasian women. Our results support the hypothesis that oxidative stress resistance pathways, which modulate lifespan in model organisms, also influence human longevity.

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No association between severe obesity and ACE gene functional variants in French Caucasians. *C. Bell[1,9], D. Meyre[2], E. Petretto[3], C. Boyle[1], B. Jouret[3], D. Jaquet[4], C. Levy-Marchal[4], M.A. Charles[5], J. Weill[6], M. Tauber[7], C.A. Mein[8], T.J. Aitman[3], P. Froguel[1,2], A.J. Walley[1].* 1) Section of Genomic Medicine, Imperial College, London, W12 0NN UK; 2) CNRS, UMR 8090, Pasteur Institute, Lille, France; 3) Section of Physiological Genomics, London, W12 0NN UK; 4) INSERM U457, Robert Debre Hospital, Paris, France; 5) INSERM, U258-IFR69, Paris South Faculty of Medicine, Villejuif, France; 6) Paediatric Endocrine Unit, Jeanne de Flandre Hospital, Lille, France; 7) INSERM U563, Childrens Hospital, Toulouse, France; 8) Barts and the London Genome Centre, Queen Marys College, London, UK; 9) Department of Haematology and Genetics, Prince of Wales Hospital, Sydney, NSW, Australia.

For the Angiotensin-Converting Enzyme (ACE) gene, associations with obesity phenotypes have been reported for the Alu insertion/deletion (I/D) polymorphism in Italians and single-nucleotide polymorphisms (SNP)/clades in Swedes. We have also previously reported a linkage result in a genome scan of severely obese French Caucasians, at chromosome 17q23, which contains the ACE gene. Eight polymorphic ACE gene polymorphisms (seven SNPs and the I/D polymorphism) were genotyped in 1054 severely obese cases (548 adults (mean BMI = 45.4), 506 children (ZBMI > 97th percentile) specifically recruited for extreme obesity and 918 non-obese, non-diabetic controls. These SNPs include those proposed as being responsible for variation in ACE levels and also enabled the previously-described clades to be inferred. Statistical analysis of the frequencies of alleles, genotypes, haplotypes, clades and diploclades showed no significant difference at all ($p>0.05$) between cases and controls in total or when age and/or sex were taken into account, after Bonferroni correction. Quantitative analysis of the clades and diploclades for BMI, waist to hip ratio, or ZBMI in children, were not significant as well. These results demonstrate that functional sequence variation in the ACE gene, at whatever level, is not associated with either severe or morbid obesity phenotypes in French Caucasian subjects.

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Characterization of the 2p dyslexia candidate region. *H. Anthoni[1], M. Zucchelli[1], J. Schumacher[2], G. Schulte-Körne[3], M. Nöthen[4], J. Kere[1,5], M. Peyrard-Janvid[1].* 1) Dept of Biosciences at Novum, Karolinska Institutet, Sweden; 2) Institute of Human Genetics, University of Bonn, Germany; 3) Dept of Child and Adolescent Psychiatry and Psychotherapy, University of Marburg, Germany; 4) Dept of Genomics, Life & Brain Center, University of Bonn, Germany; 5) Dept of Medical Genetics, Biomedicum, University of Helsinki, Finland.

Developmental dyslexia is a complex disorder characterized by unexpected difficulty in learning to read despite adequate intelligence, education, and normal senses. It is one of the most frequently diagnosed disorders in childhood, with a prevalence rate of 3-10%. Genetic loci have been mapped to at least seven different chromosomes by several independent studies. Candidate genes have so far been proposed for *DYX1* (*DYX1C1*) and *DYX2* (*VMP*, *DCDC2*, *KIAA0319*, *TTRAP*, *THEM2*). We have previously mapped a susceptibility gene for dyslexia to 2p11-p12 in eleven Finnish pedigrees using microsatellite markers. In order to refine this candidate region, we increased the marker density by successive rounds of fine mapping using microsatellites and SNPs in an extended Finnish sample set, including new families and case-controls. Initially, 29 new microsatellites narrowed the 40 cM candidate region down to 6 Mb. Three positional and functional candidate genes were screened by direct sequencing in affected individuals; *TACR1*, *CTNNA2* and *LRRTM4*. All of these have important roles in the central nervous system and are abundantly expressed in brain. However, no mutations could be detected in their coding regions. We further selected and genotyped 26 SNPs in the 6 Mb region. Association analysis using the HPM algorithm and the TDT gave significant p-values for a number of individual markers (p=0.003). We replicated these associations in an independent sample set of 253 trios of German origin, showing the same marker patterns and alleles as in the Finnish sample, with the best single marker p-value of 0.002. To refine this association and further narrow down the candidate gene(s), we are now genotyping SNPs in the region at a 10 kb density, characterizing the three positional candidate genes it contains, as well as identifying any unknown genes.

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DOCK9, a candidate bipolar disorder susceptibility gene on chromosome 13q32.3. *S. Detera-Wadleigh[1], C. Liu[2], J. Badner[2], W. Corona[1], N. Akula[1], I. Cardona[1], M. Kundu[1], C.J.M. Steele[1], M. Maheshwari[3], T. Bonner[1], R. Gibbs[3], E. Gershon[2], F. McMahon[1].* 1) NIMH/NIH, Bethesda, MD; 2) University of Chicago, Chicago, IL; 3) Baylor College of Medicine, Houston, TX.

Overlapping linkage peaks for schizophrenia and bipolar affective disorder (BPAD) span a wide expanse of chromosome 13q. A preponderance of studies highlight 13q32-q33. The G72/G30 locus, associated with schizophrenia and BPAD, accounts for some of the linkage signal in the region. In order to detect additional susceptibility genes in the region, we undertook association mapping of a ~7 Mb interval on 13q32-33. Initially, 98 SNPs were genotyped in a sample of 285 families ascertained through probands with BPAD. Family-based association analysis detected nominally-significant signals at 8 SNPs. Some of these results could be spurious, but 3 of the associated SNPs reside in the DOCK9 gene, a Rho-GTPase activator involved in axonal guidance. These SNPs cover the 5flanking region, extending to the first intron of AK127329, and are part of a ~90 kb haplotype block. One of these SNPs significantly partitioned the linkage evidence in this sample. Replication testing in a second sample of 335 families also detected significant evidence of association with BPAD. Subsequently, 16.5 kb of the associated haplotype block were re-sequenced in 24 cases, half of which were derived from 13q-linked families. This detected several novel SNPs and a 5-bp intronic deletion. The deleted allele, initially found only in 13q-linked families, was transmitted in excess to affected offspring, but no SNPs so far appear to be functional. DOCK9 may be one of the genes on 13q that contributes to susceptibility to BPAD.

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Epidemiological and genetic survey of hypertension in a Sardinian isolated population. *A. Angius[1,2], M. Fanciulli[2], M. Fattorini[2], V. Cabras[1], M. Adamo[2], D. Serra[2], D. Degli Esposti[3], G. Biino[1,2], M. Pirastu[1,2].* 1) Institute Population Genetics,National Research Council,CA,Italy; 2) SharDNA LifeScience,CA,Italy; 3) Department Internal Medicine,University Bologna,BO,Italy.

The aim of this study was estimate the prevalence of hypertension and to identify the underlying genetic factors in the general population of an isolated village of Sardinia: Talana (~1000 inhabitants). Several lines of evidence indicate that this village has clinical similarity to the European population, while it has more power for genetic analysis than outbred population. Voluntary participants (80%) of the entire population underwent blood tests and a standardized interview collecting demographic information, living habits, clinical and family history. Hypertension was defined as SBP 140 and DBP 90 mmHg or current treatment for hypertension. SBP and DBP were normally distributed with average of 13520 and 8010 mmHg respectively and 26%; of the adult population of Talana was affected by hypertension. The epidemiological survey highlighted the association of the same clinical and environmental factors which were found in different populations. Eight multigenerational pedigrees, that consisted of 332 total individuals of whom 67 were affected, were constructed. The majority of the extant Talana population was genotyped using 1054 markers, which comprehend 50% of the hypertension family members. We reconstructed a genetic map using the frequencies estimated on the entire population. GWS was performed by a non-parametric analysis using SIMWALK2. We confirmed the previously described locus on chr 2 (-log10(P):3.872) and identified 8 additional loci with suggestive significance on chr 3, 4, 10, 14, 18, 19, 20 and 22. Increasing the number of markers we confirmed the loci on chromosome 18 (-log10(P): 2,97), 20 (-log10(P):2,41) and 22 (-log10(P):4). We replicated some of results in a nearby isolated village with different founders and low genetic exchange with Talana, confirming loci on chr 18 and 20, which were previously described in different populations. We are currently investigating these loci with high density SNPs map taking advantage of the extended linkage disequilibrium in our population.

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Differential liabilities of rare coding and common non-coding *RET* mutations explain the multifactorial genetics of Hirschsprung Disease. *E. Emison, A. Rea, A. Chakravarti.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Hirschsprung aganglionosis (HSCR) is a non-Mendelian birth defect that affects four times as many males as females; mutations are known in 8 genes (*ECE1*, *EDN3*, *EDNRB*, *GDNF*, *NRTN*, *RET*, *SOX10*, *ZFHX1B*). The pathognomonic hallmark of HSCR is the absence of neural-crest cell-derived enteric ganglia along variable segments of the gut. In the mouse, several genes encoding key proteins involved in neural crest development, including those with HSCR mutations, have peak expression in the developing gut at 13.5 dpc, coincident with the peak migration of neural crest-derived neuronal precursors in the hindgut. We utilized this biological feature to identify new genes with identical features within mapped human HSCR loci to assess whether they contribute to HSCR risk.

Our family-based linkage studies have revealed three contributory loci (3p21, 9q31 and 19q12) where the disease genes have yet to be elucidated. To identify HSCR genes at 3p21, we have interrogated expression of all known and predicted genes within the orthologous region on mouse chr14. A total of 62 known and predicted protein-encoding genes were identified in the relevant 10 Mb interval. Expression profiles for each of these transcripts were determined in RNA isolated from a panel of 42 gender-identified pre- and post-natal mouse tissues. Duplicate male and female samples were analyzed for a total of 168 tissue samples. In parallel, expression profiles of 17 transcripts which encode proteins known to be involved in the development of the neural crest were determined. Patterns of temporal and spatial expression of the chromosome 3 orthologs in comparison with patterns of expression for genes known to be involved in the development and establishment of the neural crest have been used for predicting candidate genes. Further, as HSCR is more prevalent in males than females, we are assessing whether genes known to be involved in neural crest development are differentially expressed in the developing male and female embryos.

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A multi-pronged approach to association testing for body mass index in the 3q27 region, guided by prior probability. *H. Lyon[1], X. Zhu[2], R. Cooper[2], X. Wu[2], J. Drake[1,3], T. Bersaglieri[1], M. Egyud[1], J. Hirschhorn[1,3].* 1) Div of Genetics, Boston Children's Hosp, Boston, MA; 2) Dept of Prev Med and Epidemiology, Loyola Univ, Maywood, IL; 3) Broad Inst, Harvard and MIT, Cambridge, MA.

Obesity in African-Americans is increasing rapidly and is co-morbid with hypertension, diabetes and heart disease. Linkage analysis has identified the 3q27 region as an obesity QTL in African-Americans. We are using multiple approaches to test variants with the highest prior probability of being associated with BMI: SNPs in candidate genes, SNPs predicted to affect protein function, or SNPs in LD with many other SNPs in the region. Specifically, we are (1) selecting and genotyping tag SNPs in 11 candidate genes; (2) genotyping all known missense SNPs, and (3) using an LD-based approach to survey as much of the region as possible by genotyping ~1,000 of the most informative SNPs - those that are proxies for the largest number of other variants in the region. These three sets of SNPs are being tested for association by genotyping them in a cohort of 1007 African-Americans (572 women, 435 men). **For (1), candidate genes** were chosen based on involvement in a metabolic pathway: ACDC, GHSR, PLD1, TLOC1, PRKCI, SERPINI1, BCHE, SKIL, TNFSF10, GLUT2, CLDN11. To select tag SNPs for these genes, we genotyped a dense set of SNPs (1 SNP per 2 kb) in a random trio panel drawn from our African-American population. By also genotyping these SNPs in the Yoruba and CEPH HapMap samples, we found that HapMap data will be adequate to select tag SNPs that are informative in our African-American sample. **For (2), missense SNPs** were identified by applying custom annotation software to UCSC genome browser tables. **For (3), informative SNPs** for the LD approach were chosen based on LD patterns from the Yoruba cohort; we chose the SNPs that tagged the largest number of proxies based on a pairwise measure of LD (r^2). We estimate that this set of ~1,000 SNPs captures about a quarter of the estimated 50,000-100,000 common variants in the 30Mb region, and thus serves as an efficient first pass. This LD-based approach can be generally applied to complement candidate gene and missense SNP-based strategies in association studies.

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Contribution of sequence variation within the ABCG1 gene to bipolar disorder and suicidal behaviour. *S. Deutsch[1], C. Buresi[2], M.L. Samolyk[2], C. Gehrig[1], E. Neidhart[2], M. Gagnebin[1], S.E. Antonarakis[1], A. Malafosse[2].* 1) Dept of Genetic Medecine and Development, University of Geneva, Geneva, Switzerland; 2) Dept of Psychiatry, University of Geneva, Geneva, Switzerland.

Independent studies have demonstrated the involvement of a locus on chromosome 21q22.3 in the etiology of bipolar affective disorder (BPAD). Here we investigate the contribution of the ABCG1 gene, an ATP-binding cassette (ABC) transporter located within the HSA21 candidate region, in conferring susceptibility to BPAD and other related mood disorders. Our cohort for case-control association studies consisted of 330 controls and 950 affected subjects, who were diagnosed on the basis of DSM-IV criteria and included individuals with unipolar and bipolar disorder, as well as subjects with a history of suicide attempts. Six SNPs distributed across the ABCG1 gene, which were selected on the basis of pairwise LD values, were screened in all subjects. No significant association was detected when comparing genotypic distributions of control subjects versus all affected patients, or versus bipolar patients for any of the SNPs studied. However, significant associations were observed for a SNP located in the 3UTR of the gene (rs1044317) when comparing genotype frequencies of controls to subjects with a history of violent suicide attempts (p=0.005). A second SNP (rs2234719) located in intron 6 of the gene was also marginally associated to violent suicidal behaviour (p=0.05). Since no coding SNP in ABCG1 was identified, we hypothesised that associated SNPs might act by modifying the levels of ABCG1 mRNA. To test this hypothesis, we measured ABCG1 expression levels in cDNAs from lymphoblastoid cell lines of 40 unrelated individuals by Taqman qPCR. Significant differences in gene expression were observed, and Kruskall-Wallis analysis showed that SNP (rs2234719) was associated with ABCG1 mRNA levels (p=0.003). We conclude that ABCG1 polymorphisms contribute to susceptibility to violent suicidal behaviour, and that at least some of this contribution is mediated by modifying the expression of ABCG1. Additional studies are in progress to further characterise the functional nature of the sequence variants studied.

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Replicated association of a functional promoter variant at the serotonin 1A receptor locus with autism. *L. Jiang, R.J. Delahanty, J.S. Sutcliffe.* Center for Human Genetics Research, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Autism is a neurodevelopmental disorder exhibiting deficiencies in development and use of language and social interactions, in addition to patterns of repetitive, restricted and stereotyped behaviors and interests, and resistance to change in environment and daily routines. Abnormalities of serotonergic regulation have been long speculated as an etiological factor in autism given findings of a heritable elevation of circulating 5-HT in ~25% of subjects, efficacy of selective serotonin reuptake inhibitors (SSRIs) in treating repetitive, rigid and compulsive behaviors and anxiety, and increased symptom severity following tryptophan depletion. We recently showed that allelic heterogenetity at the serotonin transporter (SERT) locus (SLC6A4) suggests it represents an autism susceptibility gene. The 5-HT_{1A} receptor is another critical regulator of central serotonergic function, as it acts both postsynaptically and presynaptically as a somatodendritic autoregulatory receptor. To explore the potential for genetic risk alleles to exist at this locus, we identified and genotyped a series of single nucleotide polymorphisms (SNPs) in a dataset of 384 combined multiplex (327) and parent-child trio (57) families. Analysis of linkage disequilibrium (LD), consistent with data from the HapMap Project, revealed that this locus resides in an expansive ~500kb block of LD in 5q11.2. Tests of allelic association showed significant male-specific association at several SNPs when considering paternal transmissions. One marker represents a functional promoter polymorphism, and the associated allele ($P=0.01$) results in partial loss of transcriptional repression and has been linked to depression, suicide, poorer SSRI treatment response, and other phenotypes. An infrequent nonsynonymous variant was also associated. A replication analysis in a sample of 262 independent families revealed a more significant overall association ($P<0.005$) and a nominal parent-of-origin effect with the same markers. These results strongly suggest a role for HTR1A in genetic risk for autism spectrum disorders.

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The integrin 3 locus (ITGB3) confers genetic risk for autism. *R.J. Delahanty, L. Jiang, J.S. Sutcliffe.* Center for Human Genetics Research, Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Autism is a neurobehavioral phenotype involving abnormalities in the development of language and social relationships, and repetitive, rigid and compulsive behaviors and resistance to change. Proximal chromosome 17q is implicated as harboring significant male-biased genetic effect(s) in autism, based on linkage studies by the Autism Genetics Resource Exchange (AGRE) Consortium, the International Molecular Genetic Study of Autism Consortium (IMGSAC), and our group. Evidence for association at the serotonin transporter (SERT) locus (SLC6A4) is mixed, although increasingly reports suggest involvement of the short allele at the insertion/deletion promoter variant (HTTLPR). We recently identified a number of rare coding and noncoding variants at SLC6A4 that suggesting it is a risk locus for autism under an allelic heterogeneity model. This may explain inconsistent reports of association in the presence of highly significant linkage in this region. Careful examination of the linkage data on 17q strongly suggests that SLC6A4 is not the only main effect locus in 17q. A candidate for the second locus is ITGB3, recently shown to represent a male quantitative trait locus (QTL) for circulating serotonin (5-HT). Because 5-HT is elevated in ~25% of subjects with autism, we examined ITGB3 for evidence of contribution to genetic risk for autism. Linkage disequilibrium across the locus was evaluated, and markers representing common alleles were selected. We included a nonsynonymous (Pro33Leu) SNP, shown to associate with 5-HT levels. Analysis in a dataset of 643 predominantly multiplex families from Vanderbilt, AGRE, and other groups in the NIMH Repository revealed significant overall association to the Pro33Leu SNP (P=0.0026), but not other common alleles tested. Surprisingly, no significant association was detected when solely examining transmissions to males, or transmissions in families with only affected males. These data support a role for ITGB3 in autism susceptibility, suggest that this coding variant may exhibit dysfunctional properties, and that ITGB3 is very likely to represent the second locus contributing to highly significant linkage in proximal 17q.

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High-throughput candidate gene screening for dyslexia susceptibility genes on chromosome 18p11-q21. *T. Scerri[1], A. Morris[1], J. Stein[2], A.P. Monaco[1].* 1) Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, United Kingdom; 2) Department of Physiology, Oxford, OX1 3PT, United Kingdom.

Developmental dyslexia (DD) is a specific and significant impairment in reading ability despite adequate educational opportunity and intelligence, and is diagnosed in ~5% of school-aged children. We first reported linkage of a DD quantitative trait locus (QTL) at 18p11.2 in three large independent family samples from the UK and US, and have since identified a second DD QTL at 18q12.2. These 2 loci together cover ~40Mb of genetic material and contain over 200 genes. We have embarked on a gene-based, high-density SNP genotyping and association study to identify the DD susceptibility variants on chromosome 18.

We used genotype data of the CEPH families from the HapMap project to evaluate the distribution of linkage disequilibrium (LD) and haplotype blocks across the ~40Mb region. We selected haplotype-tagging SNPs using Haploview (with the Gabriel algorithm) from blocks overlapping all known genes and their putative regulatory elements, thereby reducing the amount of genotyping required and capturing the majority of common variation in ~80% of these gene regions. Following power calculations to maximize efficiency, we genotyped 1492 SNPs at 18p11-q21, and a further 44 unlinked SNPs for inheritance checking and stratification purposes, using the GoldenGate assay, on a sample of 960 individuals that includes 192 sex- and population-matched controls, and 188 nuclear UK families each containing a proband with severe dyslexia.

Preliminary bioinformatic analysis has been carried out on the LD block and gap sizes, and intragenic haplotype tagging efficiency. Sample and SNP genotyping success rates are both at least 97%. Analysis will be performed on haplotypes within each block using both family-based and case-control association tests, and the results presented.

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Linkage and association of SLC11A1 and tuberculosis. *C.D. Hamilton, W.F. Hulme, L. Zhang, S.G. Patillo, C.A. Linton, R.M. Carney, A.W. Mosher, J.R. Gilbert, W.K. Scott.* Dept. of Medicine, Duke Univ. Medical Center, Durham, NC.

Tuberculosis (TB) is a significant cause of premature mortality worldwide. 10% of exposed individuals develop pulmonary TB, suggesting that host factors, partly under genetic control, determine development of active TB. SLC11A1 (formerly NRAMP1) was linked to TB in a Canadian aboriginal family and associated with TB in Africans, Asians, and North American whites. SLC11A1 is an ion transporter that influences the intracellular environment, potentially affecting *M. tuberculosis* replication. We examined nine markers in SLC11A1 for linkage and association with TB in a family-based study of 477 individuals from 229 families (143 African-American and 76 white). Individuals older than 14 years with culture-confirmed pulmonary TB and children younger than 14 years with culture- or clinically-confirmed TB reported to the NC or SC TB control programs from 1992-2005 were enrolled along with unaffected sibling or parental controls. FASTLINK was used to analyze 23 multiplex families for linkage. Suggestive linkage was obtained at G249G (lod=1.7,=0). Family-based tests of association were conducted in each ethnic group using APL. Of the four markers associated with TB in other studies, only rs3731865 (intron 4) was significantly associated with TB in the African-American sample (p=0.02). In whites, two 3' UTR markers were associated with TB: rs1059823 (p=0.007) and rs13062 (p=0.04). These results are novel to this sample. None of these three SNPs has a known function. Contrary to prior studies, no association was detected with the 5'(GT)n promoter polymorphism associated with lower expression of SLC11A1, the rare coding variant in exon 15 (D543N) or the 3' TGTG ins/del polymorphism. Consistent with previous studies, markers in the 5' and 3' ends of SLC11A1 are independently associated with TB. In this sample, association in African-Americans is restricted to intron 4, and association in whites is limited to the 3'UTR. The patterns of association in this study, compared to prior studies, suggest that the functional variants in SLC11A1 that increase susceptibility to pulmonary TB remain to be discovered and may differ by ethnicity.

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Objective prioritization of positional candidate genes at a QTL for pre-eclampsia on 2q22. *E. Moses[1], E. Fitzpatrick[2], K. Freed[2], S. Forrest[3], T. Dyer[1], J. Blangero[1], S. Brennecke[2].* 1) Dept Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Dept Perinatal Medicine & University of Melbourne Dept of Obstetrics & Gynaecology, Royal Women's Hospital, Melbourne, Australia; 3) Australian Genome Research Facility, Melbourne, Australia.

Pre-eclampsia/eclampsia (PE/E) is the most common and serious disorder of human pregnancy. While the familial association of PE/E has been recognized for decades the genetics are complex and poorly understood. In an attempt to identify PE/E susceptibility genes we embarked on a positional cloning strategy using 34 Australian & New Zealand PE/E pedigrees in which the estimated heritability is 0.647 0.168. An initial 10 cM resolution genome scan revealed a putative susceptibility locus spanning a broad region on chromosome 2 that overlaps an independent linkage signal seen in Icelandic PE pedigrees. We have now genotyped an additional 25 STR markers in this region and used a variance components-based linkage approach to perform multipoint linkage analysis on this dataset. Under the strict diagnosis of PE, we obtained significant evidence of linkage on 2q with a peak LOD score of 3.43 near marker D2S151 at 155 cM. To prioritize positional candidate genes at this QTL for detailed analysis, we have applied an objective prioritization strategy that integrates quantitative bioinformatics, assessment of differential gene expression and SNP association/LD analyses. Our bioinformatics tool assigned the highest priority to the activin receptor gene ACVR2. This gene also showed greater than 10-fold differential gene expression in human decidual tissue from normotensive and PE individuals. We genotyped five known SNPs in this gene in our cohort of 34 PE/E pedigrees and performed tests for association and LD using the QTDT test. One SNP (rs1424954) showed strong preliminary evidence of association with PE ($p = 0.007$) while two others (rs1364658 and rs1895694) exhibited nominal evidence ($p < 0.05$). There was evidence of weak LD among these SNPs. The highest observed LD occurs amongst the three associated SNPs suggesting that the observed signals may be the signature of an observed functional variant.

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Cluster Rank Analysis provides evidence for mitochondrial inheritance of presbycusis. *D.L. Newman[1], K.D. Raish[1], L.E. Edsall[1], C.M. Witkowski[1], D.R. Frisina[2], P.A. Shipman[1], M.V. Osier[1].* 1) Dept Bio Sci, Rochester Inst Tech, Rochester, NY; 2) Internatl Ctr for Hearing & Speech Research, Rochester, NY.

The genetic basis of human presbycusis (age-related hearing loss) is unknown. This common disorder is characterized by inability to understand speech, particularly in noisy backgrounds. Audiograms of presbycusics show sloping hearing loss, with greatest deficiencies at the highest frequencies, and over time an individuals hearing loss progresses into the lower frequencies that are more important for understanding speech. We hypothesized that the mitochondrial genome plays a role in presbycusis susceptibility, based on previous research that showed a greater correlation of presbycusis for mothers and their children than for fathers and their children (Gates et al. 1999; Marietta et al. 2004). We sequenced the hypervariable region (HVRI&II) of the mitochondrial genome for >200 subjects aged 58 and older who were extensively characterized for their hearing abilities. We used a novel method, Cluster Rank Analysis (CRA) to explore correlations between phenotypic and genotypic data. HVRI&II sequences for each individual were taken as the independent variable, and distances between the values were used to generate a UPGMA tree. Values from each of several dependent variables (quantitative measures of hearing ability) were then assigned to the appropriate node of the tree. Phenotypes included pure tone thresholds from 500 to 20,000 Hz, pure tone averages (PTA1-4), speech reception thresholds, and otoacoustic emissions (DPOAE, TEOAE) for each ear, as well as binaural speech comprehension. The identical tree was identified in which the ranks, from left to right, were ordered to maximize a rank statistic (e.g. Spearman Rank Correlation). The rank statistic for each dependent variable was determined and the list of variables sorted based on the rank statistic. We found that the high frequency thresholds were strongly correlated with position on the tree (Spearman correlation of 0.72-0.88). This result supports the hypothesis that a mitochondrial factor influences the development of presbycusis. Future studies will follow up on mitochondrial lineages to identify causative mutations.

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Oxidative stress candidate genes and mitochondrial haplogroup analyses in multiple sclerosis. *J. Hart[1], J.M. van der Walt[1], J. DiPiero[1], J. Rimmler[1], S. Schmidt[1], S. Gregory[1], N. Schnetz-Boutaud[2], S.J. Kenealy[2], M.A. Pericak-Vance[1], J.L. Haines[2].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Reactive oxygen species (ROS)generated within brain tissue can lead to the damaging oxidation of lipids, proteins and DNA. Accumulation of this damage (oxidative stress) can lead to disease pathogenesis if cells are unable to eliminate ROS agents through the antioxidant defense system. Several lines of evidence have implicated oxidative stress and mitochondrial dysfunction in the etiology of multiple sclerosis (MS). We tested whether mtDNA SNPs or nuclear SNPs within oxidative response genes influence MS risk. We genotyped 10 SNPs that define the Caucasian haplogroups in cases (n=469) and age-matched Caucasian controls (n=463). We did not observe any significant difference of any mtDNA allele frequency or haplogroup between cases versus controls. We then selected a set of oxidative stress candidate genes to test for association with MS risk which included: solute carrier 11A1 (SLC11A1), heme oxygenase 1 (HMOX1), glutathione reductase 1&2 (GSTO1,GSTO2). In this preliminary analysis, 5-7 SNPs were genotyped in 842 families and 636 discordant sib-pairs. Family-based tests (PDT, APL) were used to test whether SNPs in these genes influence risk of MS overall or in subsets stratified by HLA-DR2pos or HLA-DR2neg. Both APL and PDT results demonstrated that one SNP(rs743811) within HMOX1 influences risk of MS in the DRpos; set (p=0.03). All other SNPs tested were not significant. However, a second SNP (rs73777) located in an adjacent gene (MCM5)also showed significant association with MS risk in the overall dataset (p=0.01). These two SNPs were in strong linkage disequilibrium (D=0.99). Our results indicate that Caucasian haplogroup defining SNPs do not influence risk of MS. However, two markers in strong LD located in HMOX1 and MCM5 genes influence risk of MS in our family-based study. Since HMOX1 is upregulated in brain tissue under oxidative stress, we intend to pursue this candidate gene for further study.

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MTND1*LHON4216C, a secondary Lebers Hereditary Optic Neuropathy polymorphism, and age-related macular degeneration (AMD): A report of an age-matched case-control study. *J.A. Canter[1], M.A. Pericak-Vance[2], M.A. Hauser[2], S. Schmidt[2], W.K. Scott[2], P. Gallins[2], K.L. Spencer[1], S.Y. Kwan[2], M. Noureddine[2], J.R. Gilbert[2], N. Schnetz-Boutard[1], A. Agarwal[1], E.A. Postel[2], L.M. Olsen[1], J.L. Haines[1].* 1) Vanderbilt University Medical Center, Nashville, TN; 2) Duke University Medical Center, Durham, NC.

Age-related macular degeneration (AMD) is the leading cause of severe, irreversible vision loss in white persons over age 50. MTND1*LHON4216C alters a key subunit of Complex I in the mitochondrial electron transport chain and has been implicated in several neurodegenerative disorders, including an association with Leber's Hereditary Optic Neuropathy. We hypothesized that this variant is also risk factor for AMD. While age is a key risk factor in the development of AMD, the MTND1*LHON4216C polymorphism may also impact longevity either directly or via linkage with other mitochondrial polymorphisms. Due to the importance of age in predicting risk of AMD, we devised a matching strategy that matched on age at time of examination and optimized inclusion of cases and controls. Among 246 extensively evaluated cases (defined as stage 3, 4, or 5 AMD) and 116 controls, we age- and race-matched 103 pairs of subjects (exclusively white) for this analysis. Of these pairs, 74 (72%) were matched on the exact age at time of examination. The mean age at examination for the group was 67.9 years. Cases were on average 3 years older than controls. Fifteen of 103 (14.6%) had the MTND1*LHON4216C variant, while only 5/103 (4.9%) of controls had this variant (OR 3.34, 95% CI 1.09-12.17, p=0.02). Cases and controls were not statistically different with regard to age at examination, gender, or cigarette smoking. MTND1*LHON4216C remained an independent predictor of AMD after adjustment for both complement factor H (CFH) Y402H status and smoking status using logistic regression analysis (OR 3.02, 95% CI 1.04-8.73). No interaction was observed between MTND1*LHON4216C and the CFH Y402H variant. These results suggest a role for the MTND1*LHON4216C variant in susceptibility to AMD directly or by its association with other polymorphisms in the TJ mitochondrial cluster.

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Endostatin T48 variant has an impaired affinity to basement membrane components. *O.T. Suzuki[1,2], H. Tu[3], T. Pihlajaniemi[3], P.H. Godoi[4], G. Oliva[4], B.R. Olsen[5], M.R. Passos-Bueno[1,2].* 1) Centro de Estudos do Genoma Humano; 2) Instituto de Biociências, Universidade de São Paulo, Brazil; 3) Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu; 4) Instituto de Física de São Carlos, USP, São Carlos; 5) Department of Cell Biology, Harvard Medical School, Boston.

Angiogenesis is a process involved in numerous diseases, such as cancer, rheumatoid arthritis and diabetic retinopathy. Endostatin (ES), a proteolytic cleavage product of the c-terminal domain of type XVIII collagen, is a potent angiogenesis inhibitor that influences endothelial cell proliferation, migration, apoptosis and tube formation. It is an adhesion molecule that binds to several extracellular matrix (ECM) components. Lack of type XVIII collagen causes Knobloch syndrome (KS), an autosomal recessive condition characterized by high myopia, macular abnormalities, vitreoretinal degeneration, retinal detachment and occipital encephalocele. We have suggested through molecular modeling that the polymorphic ES residue change D104N (D1437N) might alter its function and predispose to prostate cancer (2001, *Cancer Res*, 61:7375). Another rare residue change in the ES domain, A48T, was found in homozygosity in only two KS sib patients (Kliemann *et al*., 2003, *Am J Med Genet A*, 119:15) and its relation to the disease is still unknown. We produced recombinant human ES N104 and T48 to evaluated their binding properties to other ECM proteins using ELISA and surface plasmon resonance. Fibronectin, type IV collagen, nidogen, heparin-BSA, laminin-1-nidogen-1 complex, perlecan, and fibulin were tested as ligands. N104 ES did not show a great difference from the wild type protein in its binding to the tested molecules, while the T48 ES revealed a slightly lower but significant affinity to the laminin-1-nidogen-1 complex and fibulin ($P = 0.0017$ and $P = 0.0011$, respectively). Based on the protein interactions studied, the A48T change seems unlikely to result in KS, unless it alters the interaction with a still unknown protein. On the other hand, N104 ES does not seem to significantly impair the function of the protein. FAPESP/CEPID, CNPq.

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Alternative Splicing and Sequence Variation in *NRG3*, a Positional Candidate for Schizophrenia. *N. Feng, S. Almashanu, D. Avramopoulos, C. Obie, K. Cheng, M.D. Fallin, A.E. Pulver, D. Valle.* Johns Hopkins School of Medicine, Baltimore, MD.

Neuregulin 1 (*NRG1*) located at 8p22-21 has been implicated in schizophrenia (SZ) in genome-wide scan studies and by studies of an *NRG1* knockout mouse model. NRG1 encodes a single membrane pass plasma membrane protein with an EGF motif in its extracellular N-terminus. Interestingly, an *NRG1* paralog, *NRG3*, maps to 10q22, a region suggested to harbor a SZ susceptibility gene by our linkage studies in Ashkenazi Jewish (AJ) families with SZ (Fallin et al Am J Hum Genet 73: 601, 2003). *NRG3* has structural similarity with *NRG1* with 12 exons distributed over 1.2 Mb of genomic DNA but unlike *NRG1*, its expression is limited to the CNS. To learn more about this positional candidate for SZ, we characterized splice forms of the human *NRG3* and performed molecular screening for sequence variants of *NRG3* in AJ SZ families. Using RT-PCR and subsequent sequencing as well as blast searches of the EST database, we found that *NRG3*, like *NRG1*, has at least 6 splice forms and multiple 5 transcriptional start sites that produce different N-terminal sequences. Interestingly, in both genes, a highly conserved micro exon 3 of two tandem acceptor splice sites in intron 3 encodes a segment of the protein that lies just on the extracellular side of the transmembrane domain, a location that may be important for release of the EGF domain by proteolytic cleavage. Additionally, we sequenced all 12 exons plus 5 conserved non-coding regions and 1000 bp of 5 UTR and proximal promoter of *NRG3* in 15 AJ SZ probands and 6 controls. We identified 6 single nucleotide substitutions including 5 known SNPs (rs478010, rs17101139, rs17101193, rs17101196 and rs2295933) and one novel SNP in the proximal promoter. Of the known *NRG3* SNPs, 2 are synonymous and one is nonsynonymous variant (rs17101193, N576K). We are proceeding to genotype these variants in our collection of approximately 600 AJ SZ patients and 600 AJ and to determine if there is developmental and/or region-specific expression of the *NRG3* splice form.

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Mutations in the FGF/FGFR Pathway Play a Role in Nonsyndromic Cleft Lip and Palate. *B. Riley[1], M. Mansilla[1], B. Maher[2], B. Trump[1], E. Russo[1], A. Vieira[1], M. Marazita[2], J. Murray[1].* 1) University of Iowa; 2) University of Pittsburgh.

Isolated or nonsyndromic cleft lip and palate (NS CLP) is a complex multifactorial birth defect resulting from a combination of genetic and environmental factors. We are investigating the contribution of the fibroblast growth factors (FGF) and their receptors (FGFR) to craniofacial development in humans based on their known role in other vertebrate species and hypothesize that mutations in members of this pathway contribute to NS CLP. The following genes have been investigated: FGFR1, FGFR2, FGFR3, FGFRL-1, FGF2, FGF3, FGF4, FGF7, FGF8, FGF9, FGF10, and FGF18. We have used both direct sequencing of the coding regions of these genes in 180 NS CLP patients and linkage/association analysis using 230 multiplex Filipino families. Several likely disease-causing mutations were identified by sequencing, including a nonsense mutation, R609X, identified in a two-generation family from Iowa originally diagnosed with NS CLP. Further contact with this family revealed that the proband developed features of Kallmann syndrome but her father, who also has the R609X genotype, has NS CLP as his only phenotype. Two missense mutations in FGFR1, M369I and E467K, and one missense mutation in FGFR2, R84S, were found in five independent families but were absent in 1400 controls. A missense mutation, D62H, was identified in FGF8, which arose de novo in a child with NS CLP. Finally, in two families with confirmed parentage an obligate loss of an allele was observed for FGF4 suggesting the presence of microdeletions. Association methods have also been used to assess FGF involvement. Genotyping of single nucleotide polymorphisms (SNPs) in the FGF and FGFR candidate genes found significant P-value associations for FGF3, FGF10 and FGF18 with NS CLP. Statistical evidence also supports a gene x gene interaction between FGF10 and FGF18. In the aggregate these complementary methods, direct sequencing and statistical association, support the contribution of the FGF signaling pathway to NS CLP and support additional investigations looking at conserved regulatory regions and evidence for gene x gene interactions to fully establish their role in disease gene etiology.

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Excess of structural variants in the neurexin 1b gene in patients with autism. *J. Yan[1], J. Feng[1], A. Bockholt[1], C. Yang[1], W. Song[1], E. Cook, Jr.[2], C. Skinner[3], R. Schroer[3], C. Schwartz[3], S. Sommer[1].* 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) Dept Psychiatry, University of Chicago, Chicago IL; 3) J.C. Self Research Institute, The Greenwood Genetic Center, Greenwood, SC.

Autism is a complex developmental disorder that is characterized by deficits in social interaction and communication and by restricted and stereotyped patterns of behavior. Neuroligins are postsynaptic membrane cell-adhesion molecules which bind to b-neurexins, a family of proteins that act as neuronal cell surface receptors. Recently, mutations in the neuroligin 3 (NLGN3) and neuroligin 4 gene (NLGN4) were identified in patients with autism or mental retardation. To explore the possibility that structural variants in the b-neurexin genes could predispose to autism, the coding regions and associated splice junctions of three b-neurexin genes were scanned with DOVAM-S (Detection of Virtually All Mutations-SSCP) in 24 Caucasian patients with autism. In addition, segments of the neurexin 1b gene were sequenced in 229 additional Caucasian patients with autism. Four putative structural variants (two missense variants and two in-frame insertions) were identified in the neurexin 1b gene in six Caucasian patients with autism and not in 291 healthy Caucasian controls (6/253 vs 0/291, P=0.01). Initial family data suggest that incomplete penetrance may occur. In addition, no structural variant was found in the neurexin 2b gene and one rare structural variant was found in the neurexin 3b gene. In the context of all available data, we conclude that mutations of the neurexin 1b gene may contribute to autism susceptibility.

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Serotonin transporter polymorphism and SSRI-induced nausea. *E. Peet[1], S.B. Manuck[1], R.E. Ferrell[2], M.F. Muldoon[1], J.D. Flory[1].* 1) Psychology, Univ Pittsburgh, Pittsburgh, PA; 2) Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Background: Recent studies have examined possible associations between serotonin regulating polymorphisms, including common variation in the promoter region of the serotonin transporter gene (5-HTTLPR), and incidence of side effects in patients treated clinically with selective serotonin reuptake inhibitors (SSRIs). Here, we investigated the association between 5-HTTLPR genotypes and nausea induced by acute SSRI administration in a non-patient sample. Methods: 232 community volunteers (137 male, 95 female); ages 30-55 (msd: 45 6.5) were administered intravenously a weight-adjusted dose of the SSRI Citalopram (.33mg/kg lean body mass). Subjects reported presence or absence of nausea at t = +30, +60, +90, and +150 minutes post-infusion. Chi-square tests were used to compare nausea+/- groups by genotype of the 5-HTTLPR length polymorphism [(long/long + long/short) vs short/short alleles]. Plasma Citalopram levels were measured at t = +30, +45, and +90. Results: The highest incidence (n = 52) of nausea occurred at t = +60. Mean plasma Citalopram levels did not differ between nausea +/- groups at t = +30(p = .43), +45(p = .69), or +90(p = .83), nor did Citalopram levels vary significantly by genotype at t = +30(p = .72), +45(p = .34), or +90(p = .40). 5-HTTLPR genotypes did not differ in frequency by sex (p = .92). Nausea tended to be reported by a greater proportion of subjects carrying any long allele, relative to short/short homozygotes (25% any *l* vs. 14% *s/s*), (p = .08). Because more women reported nausea than men (p = .03), sex-specific analyses were also conducted. No effect of the 5-HTTLPR genotype was found in men, whereas nausea was reported in a significantly greater proportion of women carrying the *l* allele group (35% any *l* vs. 13% *s/s*), (p = .047). Conclusions: In women, but not men, nausea is more likely to occur in individuals carrying either one or two copies of the 5-HTTLPR *l* allele 60 minutes after administration of IV Citalopram. These findings may have implications for treatment adherence to commonly prescribed reuptake inhibitors. Supported by NIH Grants HL-40962 and HL-65137.

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Genome-wide scan for genetic markers associated with severe cutaneous adverse reactions. *S.I. Hung[1], W.H. Chung[1, 2], W.H. Fan[1], C.H. Chen[1], C.S.J. Fann[1], J.Y. Wu[1], Y.T. Chen[1].* 1) Inst. Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Dermatology, Chang Gung Memorial Hospital, Taipei, Taiwan.

Carbamazepine (CBZ) and allopurinol are commonly prescribed medication for seizure and gout/hyperuricemia, respectively. They are also frequent causes of severe cutaneous adverse reactions (SCAR), which include the drug hypersensitivity syndrome (HSS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN). Despite the treatment, these adverse events still carry significant morbidity and mortality. Using candidate gene approach, we previously identified genetic markers strongly associated with CBZ-induced SJS/TEN and allopurinol-SCAR; specifically, HLA-B*1502 is associated with CBZ-SJS/TEN (Nature, 428:486, 2004) and HLA-B*5801 with allopurinol-SCAR (PNAS, 102:4134-9, 2005). In this study, we carried out a genome-wide scan using Affymetrix GeneChip Human Mapping 100K set in a case-control association study to identify additional markers/susceptibility genes other than HLA-B molecules which might predispose these individuals to the SCAR. Using 56 cases/54 controls for CBZ-SJS/TEN and 60 cases/89 controls for allopurinol-SCAR, we confirmed the previous observation that the most significant association was observed in the SNPs of the HLA-B regions on chromosome 6 (p value for allopurinol-SCAR: 9.28x10(-9); p value for CBZ-SJS/TEN: 6.91x10(-13)). In addition, we found clustering of SNPs located between cysteine and tyrosine-rich 1 (CYYR1) gene and a disintegrin and metalloprotease with thrombospondin motifs-1 (ADAMTS1) gene on chromosome 21 were strongly associated with allopurinol-SCAR (p value=9.87x10(-6)). These genetic markers provide a plausible basis for the development of such a test to identify individuals at risk for these life-threatening conditions induced by drugs, as well as point out the direction of research for an increased understanding of the pathogenesis of these clinical syndromes.

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Investigation of serotonin related genes in antidepressant response. *E.J. Peters[1], J.B. Kraft[1], S.L. Slager[2], G.D. Jenkins[2], M.S. Reinalda[2], P.J. McGrath[3], S.P. Hamilton[1].* 1) Department of Psychiatry, University of California, San Francisco; 2) Division of Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) New York State Psychiatric Institute and Columbia University.

The large inter-individual variability seen in patient response to antidepressant medication is thought to be influenced at least in part by DNA variation. Selective Serotonin Reuptake Inhibitors (SSRIs) act by antagonizing the serotonin transporter, however, the delay in therapeutic response suggests other mechanisms or regulatory events are necessary for a response phenotype to occur. In a previous pilot study in a patient population with major depression (N=96) taking the Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine, we investigated 93 publicly available SNP markers in six serotonin pathway genes (HTR1A, HTR2A, HTR2C, TPH1, TPH2 and MAOA). Variants in the 5 region of TPH1 were associated with categorical response and variants in HTR2A and TPH2 were associated with response specificity (p=0.02-0.04, OR=2.3-2.9). In order to uncover unknown variants and select maximally informative tagging SNPs, we resequenced all exons, intron-exon boundaries, and 5 conserved non-coding sequence of these six genes in our pilot population. Based on the linkage disequilibrium patterns of these SNPs (N=188), we chose 46 tagging SNPs in these six genes, which are currently being genotyped in a larger study population (N=1,953) of patients with major depression taking the SSRI citalopram. This population is a subset of a larger study investigating treatment options for patients with major depression (STARD). Genotyping is being performed using a combination of single base extension with fluorescence polarization detection (FP-TDI) and multiplexed oligonucleotide ligation assay. Our initial results indicate that the His-Tyr variant in the HTR2A gene is not associated with categorical response to citalopram (p>0.1). Results from the other tagging SNPs in these six genes will be presented, as well as data from additional serotonin pathway genes utilizing tagging SNPs selected from HapMap data.

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Preliminary Pharmacogenetic Analysis of Gene Expression in Major Depressives Treated with Citalopram. *F. Mamdani[1], A. Sequeira[1], J. ffrench-Mullen[2], B. Moltrecht[1], S. Wilder[1], G. Turecki[1].* 1) McGill Group for Suicide Studies, Douglas Hospital Research Centre, McGill University, Montréal, Quebec, Canada; 2) GeneLogic Inc., Gaithersburg, Maryland, USA.

Major depression (MD) is a psychiatric disorder that affects 5-10% of the population and is considered the second leading cause of disability by the World Health Organization. Several studies have indicated the involvement of genetic factors in MD, which may also play a role in a patients response to antidepressant treatment. In order to identify gene targets that may mediate response to Citalopram (CIT) treatment, a commonly used SSRI (selective serotonin reuptake inhibitor) antidepressant, we are conducting a large-scale gene expression study of patients being administered CIT for 8 weeks, with Affymetrix U-133 microarrays being performed pre- and post-treatment. We are presenting preliminary results on 13 cases. Outlier detection was performed using several quality control variables and principal component analysis. Differential expression analysis resulted in 297 genes having altered expression between pre and post-treatment samples. Interestingly, our preliminary analysis indicates the possibility of a gender effect, which is not surprising given gender differences in MD prevalence rates. Gene ontology analysis of differentially expressed genes revealed several biological processes being affected by treatment; these include genes involved in regulation, biosynthesis and metabolism of macromolecules and proteins, as well as, transcription and response to stress. Our results suggest that there appears to be differential expression following CIT treatment and these differences may be correlated to treatment response.

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A retrospective pharmacogenetic analysis of polymorphisms in the OATP-C gene in XUO320 ALERT trial. *J.Z. Gu[1], E. Leroy[1], C. Hurwitz[1], G. Bermann[2], M. Bortolini[2], H. Holdaas[3], B. Fellström[3], A. Jardine[3], J. Meyer[1].* 1) Biomarker Development, Novartis Pharmaceuticals, Cambridge, MA; 2) Clinical Research, Novartis Pharmaceuticals, Basel, Switzerland; 3) Rikshospitalet, Oslo, Norway.

Statins are highly efficacious in lowering plasma levels of low-density lipoprotein cholesterol (LDL-C). Large scale clinical trials have demonstrated significant inter-individual variability in LDL-C reduction in response to statin treatment. Recently a pharmacogenetic effect has been demonstrated for variations in 3-hydroxy-3 methylglutaril coenzyme A reductase (HMGCR, Chasman et al., 2004), with an association between several SNPs in that gene and efficacy of pravastatin therapy. As the hepatic uptake of statins is at least partially dependent on a membrane transporter OATP-C, we postulated that genetic variation in OATP-C might impact statin bioavailability and therefore influence drug response. Fluvastatin is a synthetic competitive inhibitor of HMGCR for the treatment of hypercholesterolemia. A retrospective pharmacogenetic analysis was conducted in an attempt to evaluate and replicate observed associations between a genetic variation in the OATP-C gene (Slc21A6) and cholesterol parameters in response to fluvastatin in the XUO320 ALERT clinical trial. Specifically, we examined 6 polymorphisms in this gene. Significant associations were seen between A463C (Pro155Thr) in OATP-C and LDL cholesterol and total cholesterol reduction in response to fluvastatin treatment ($p=0.0034$), as reported from the FAME study by Dr. Carrie et al (manuscript in preparation). The LDL-C reduction for the three genotypes are 34% (AA), 23% (AC) and 28% (CC) respectively. To help explain the reason of the heterozygotes having the lowest LDL-C reduction, an analysis of interaction between the use of cyclosporine and LDL-C reduction was carried out. The results did not indicate any association between the use of cyclosporine defined either as dose, trough concentration, or the ratio of dose to trough concentration, and genotype. Moreover, the inclusion of use of cyclosporine as a variable in a model of LDL-C by genotype did not appear to be associated with the observed effect on LDL-C.

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CYP2D6 genotype and phenotype determination in a Mexican Mestizo population. *M. Lopez[1], M.E. Alonso[2], J. Guerrero[2], H. Jung[2].* 1) Sistemas Biol, Univ Autonoma Metropolitana, Mexico DF, Mexico; 2) National Institute of Neurology and Neurosurgery Manuel Velasco Suarez, Mexico DF, Mexico.

Objective: Although CYP2D6 genetic polymorphism plays an important role in interindividual and interethnic variability in drug response, very few pharmacogenetic data are available from Hispanic populations, including Mexicans. For this purpose, this study was undertaken to determine CYP2D6 genotype and phenotype in a healthy Mexican Mestizo population. Methods: Two hundred and forty three healthy Mexican Mestizo individuals were genotyped for five CYP2D6 mutant alleles by PCR-RFLP, and duplicated CYP2D6 alleles by long-PCR. Of these, one hundred subjects were also phenotyped using dextromethorphan as the probe drug. Results: The frequency of CYP2D6*2, *3, *4, *10, *17 and duplicated CYP2D6 alleles among 243 genotyped subjects was 21.4%, 1.5%, 11.5%, 14.2%, 2.1% and 6.2%, respectively. Among the 100 phenotyped subjects, we identified ten (10%, 95% confidence interval of 4.12-15.9) individuals as poor metabolizers by using the published antimode for Caucasians. The mean log10 dextromethorphan/dextrorphan ratio of the total sample was -2.05. Of the ten poor metabolizers who were also genotyped, four were homozygous for nonfunctional alleles, and six were heterozygous for CYP2D6 normal and mutant alleles. Conclusions: The population frequency of the CYP2D6 poor metabolizer status in the Mexican Mestizo population studied was estimated to be 10%, which is very similar to Spanish Caucasians. The observed frequency of the CYP2D6 alleles tested was unique for the Mexican Mestizo sample analyzed, and in accordance to the Caucasian, Asian and African admixture in this population. A good correlation between phenotype and genotype was obtained only in poor metabolizers.

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Association of the pro-melanin concentrating hormone (PMCH) gene with the body mass index as a side effect of the antipsychotic olanzapine. *Y.C. Chagnon, C. Mérette, J. Lamarche, R.H. Bouchard, M-A. Roy, M. Maziade.* Dept Molecular Psychiatry, Robert-Giffard Research Ctr, Beauport, PQ, Canada.

Introduction.We have reported a linkage at 12q24 with a chronic phenotype of obesity under antipsychotics close to the pro-melanin-concentrating hormone (PMCH) gene. We have tested PMCH for a possible association in humans with the body mass index (BMI; kg/m2) under antipsychotics. **Methods**. A search for single nucleotide polymorphisms (SNP) in the exons/introns of PMCH, and in the 5 and 3 regions has been done in the dbSNP database of the National Center for Biological Information. Two potential intronic SNPs (rs10860843, rs11558437) did not varied in our sample, while SNPs rs7973796 located 5 and rs11111201 at 3 of PMCH showed variation. These SNPs were genotyped in 300 chronically antipsychotic-treated schizophrenic (SZ) patients and 150 controls (CTL). Obese (OB; BMI30 kg/m2), overweight (OW; 25BMI30 kg/m2), and normal weight (NW; BMI25 kg/m2) SZ and CTL were compared for genotype and allele distributions of SNPs by a chi-square analysis, and for mean effect of genotypes on BMI by a variance analysis with age and sex as covariates. **Results**.No significant difference in genotype or allele frequencies were observed between SZ and CTL, or between OB, OW and NW in SZ, while rs7973796 showed a greater frequency (p=0.02) of the common allele in NW of CTL. In variance analysis, age and sex showed no effect on BMI in SZ (0.17p0.82) in contrast to CTL (0.0002p0.01). In SZ taking olanzapine, common homozygote genotype showed a higher BMI for rs7973796 (p=0.05 Duncan's Multiple Range Test) and rs11111201 (p=0.03), in contrast to SZ taking risperidone (p=0.51, 0.62, respectively), or CTL (p=0.35, 0.89, respectively). **Conclusion**.These results suggest that the common alleles of PMCH rs7973796 and rs11111201 SNPs are associated with a greater BMI in olanzapine-treated SZ, and with leanness for rs7973796 in CTL.

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Male restricted genetic association of variant R620W in PTPN22 with psoriatic arthritis. *U. Huffmeier[1], H. Burkhardt[2], J. Lascorz[1], B. Bohm[2], J. Lohmann[3], J. Wendler[4], H. Traupe[5], W. Küster[6], A. Reis[1].* 1) Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany; 2) Inst. for Clinical Immunology and Rheumatology, Dept. of Internal Medicine III, University Erlangen-Nuremberg; 3) Psoriasis rehabilitation hospital, Bad Bentheim; 4) Rheumatologische Schwerpunktpraxis, Erlangen; 5) Dept. of Dermatology, University of Münster; 6) TOMESA Clinics, Bad Salzschlirf.

Protein tyrosine phosphatase N22 (PTPN22) is a key regulator of TCR signalling in memory/ effector T lymphocytes. A PTPN22 missense variant (R620W) has been associated with several autoimmune diseases including rheumatoid arthritis (RA). Psoriatic arthritis (PsA) is an inflammatory arthritis that can accompany the skin affection in the common inflammatory disease psoriasis. The observation of phenotypical overlap between RA and PsA and the understanding of psoriasis as a T cell mediated disease lead us to investigate this variant in PsA, as well. We performed a case control study with 375 PsA patients recruited by board certified rheumatologists and 376 healthy controls. When compared to controls no association with susceptibility to PsA was detected. Similarly, association was neither evident when stratifying PsA patients for HLA-Cw6 associated risk allele, nor for clinical criteria such as poly-/ oligoarthritis, erosive joint changes and spinal involvement. In contrast, the proportion of male PsA patients carrying *620W was significantly higher than those in the *620R wild-type group ($p = 0.001$). This difference remained significant even after Bonferroni correction for multiple testing (6 tests, $p = 0.006$). Recently, when using a similar stratification strategy in RA patients, Orozco et al. (2005) also detected a sex specific trend, with males being more prone to carry the risk allele. When we stratified a group of 375 psoriasis vulgaris patients without signs of arthritis we did not find a sex difference regarding association to *620W indicating specificity for joint manifestation in psoriasis. If verified these findings are suggestive of sex-specific effects of *620W on arthritic disease susceptibility.

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High affinity nicotine receptor genes in schizophrenia and smoking. *S.H. Voineskos, V. De Luca, N. King, G. Wong, J.L. Kennedy.* Department of Psychiatry, CAMH, Clarke, Toronto, Ontario, Canada.

A number of studies have suggested that polymorphisms in nicotinic receptor genes (CHRNA7, CHRFAM7A, CHRNA4, CHRNB2) in particular the D15S1360 in CHRNA7, are associated with schizophrenia. Schizophrenic patients have a high rate of smoking, usually 75 to 85%. It has been suggested that smoking may be an attempt by schizophrenia patients to self-medicate (Adler et al, 1998), and various studies have explored the possible links between schizophrenia and smoking. The aim of this study is to investigate the role of CHRNA4 and CHRNB2 genes in conferring risk for smoking in schizophrenia. Our sample consisted of 177 DSM-IV patients affected by schizophrenia from the Toronto area. Current smoking status was assessed by medical history questionnaire, and there were 108 smokers and 69 non-smokers. There was no difference in age or ethnicity between the two groups. Our results do not demonstrate a significant association between the CHRNA4 ($p=0.142$) and CHRNB2 ($p=0.994$) genes and schizophrenia smoker versus non-smoker status. On the other hand, we found that male gender is a risk factor for smoking in schizophrenics (chi-sq=6.275; 1df $p=0.012$). Our negative results in genetic analysis might be confounded by the gender effect in our sample. Further study into the relationship between schizophrenia and smoking is warranted and additional markers in CHRNA4 and CHRNB2 genes will be typed to analyse these genes in detail as risk factors for smoking.

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Variation in low-density lipoprotein receptor-related protein 5 (LRP5) gene is associated with bone mineral density in men and women. *Y.H. Hsu[1], T. Niu[2], H. Terwedow[1], X. Xu[1], Y. Feng[1], M. Bouxsein[3], X. Xu[1], C. Rosen[4], N. Laird[5], J. Brain[1].* 1) Prog Population Genetics, Harvard Sch Pub Hlth, Boston, MA; 2) Division of Preventive Medicine, Dept. of Medicine, Brigham and Women Hospital, Harvard Medical School, Boston, MA; 3) Beth Israel Deaconess Medical Center, Boston, MA; 4) Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME; 5) Dept.Biostatistics, Harvard Sch Pub Hlth, Boston, MA.

Objective: To determine the contribution of polymorphisms in the low density lipoprotein receptor-related protein 5 (LRP5) gene to bone mineral density (BMD), and to estimate the risk of osteoporosis. Design: A population-based cohort with 1120 extreme low hip BMD cases or extreme high hip BMD controls was genotyped for 10 SNPs. We further confirmed the results by genotyping 266 offspring and 309 available parents from 160 nuclear families. A family-based association test was used. Haplotype association analysis was performed in both study cohorts. Results: Subjects with the GG genotype of the Q89R polymorphism had a 4.4 times higher risk ($p = 0.009$) of having extreme low hip BMD, and an 18.9 times higher risk ($p<0.0001$) of developing osteoporosis. Significant associations were found in men, premenopausal and postmenopausal women. Subjects with the TT genotype of the A1330V polymorphism had a 3.8 times higher risk ($p = 0.002$) of developing osteoporosis. The association between the Q89R polymorphism and BMD was further confirmed by a family-based association study ($p<0.0001$) and cross-sectional study. Individuals with haplotype G-T-T-C (Q89R - N740N - A1330V - D1363D polymorphism) had a higher risk of extreme low hip BMD and osteoporosis. Conclusions: Using both population-based and family-based association designs, our study demonstrates that genetic variation in the LRP5 gene is significantly associated with BMD in adult men, premenopausal women and postmenopausal women. Polymorphisms in the LRP5 gene are potential risk factors for osteoporosis and also potentially contribute to the normal variance in BMD in healthy adults.

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Adipokine gene variants associated with Type 2 Diabetes in the aboriginal Sakha (Yakut) population of Eastern Siberia. *N. Sambuughin[1], F. Platonov[2], P. Ignatiev[2], V. Osakovskiy[2], T. Sivtseva[2], A. de Bantel[1], A. Shatunov[1], V. Krivoshapkin[2], L. Goldfarb[1].* 1) NINDS/NIH, Bethesda, MD; 2) Institute of Health, Yakutsk, Russia.

Adipose tissue produces a variety of bioactive peptides collectively named adipokines; genetic variations in some of the adipokines profoundly affect insulin sensitivity, glucose and lipid metabolism. We studied the frequency of Type 2 Diabetes (T2D)-associated adipokine alleles in an aboriginal Sakha population of Russian Siberia, in which the T2D prevalence has increased 4-fold within the past ten years. The obtained allele frequencies were compared with published data on other Asian populations (Japanese, Chinese, Koreans), Caucasians (French, Dutch, Swedish, Finns), and Africans. The sequence variants within five adipokine genes were screened among 130 Sakha individuals. The H variant at the Y111H amino-acid-altering-polymorphism of Adiponectin was identified in 11.9% of the Sakha vs. 0 in other Asian populations and 0-1.6% in the Caucasians. The C allele of Interleukin 6 (IL6) at the minus 572 G>C polymorphic site of the promoter was detected in 42.5% of the Sakha, while it was present in 25% of the Koreans, 6.7% of Caucasians and 9.5% of the tested Africans. Tumor necrosis factor alpha (TNF) A variant at the minus 308 G>A site of the promoter was found in 16.7% of the Sakha, 1.1 to 7.1% of other Asians, but 18.0 to 22.1% of the studied Caucasians. The frequency of the T2D-associated T variant at position 156 of Resistin is less frequent in the Sakha (13.9%) than the Caucasians (21-23%). Similarly, the A allele at the minus 2549 A>C polymorphic site of the Leptin promoter was seen in 24% of the Sakha and 25.3% of the Chinese, while it was detected in 46.3% of the French population. These important differences in the spectrum of the diabetogenic alleles are likely to influence the genetic susceptibility to T2D and the effectiveness of the existing therapies.

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Association of Polymorphisms and Haplotypes in the Glucocorticoid Receptor with Chronic Fatigue Syndrome.

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Chronic Fatigue Syndrome (CFS) is a significant public health problem of unknown etiology with no identifiable lesions, diagnostic markers or risk factors. Some studies have indicated that deregulation of immune functions and hypothalamic pituitary adrenal (HPA) axis may be associated with CFS. Family, twin, and case-control studies support a genetic contribution to CFS. Based on these observations, we hypothesize that sequence variations in genes regulating immune functions and HPA axis are significant risk factors of CFS. Since glucocorticoid receptor is a major effector of HPA axis through cortisol signaling, we examined the association of sequence variations in glucocorticoid receptor gene (NR3C1) with CFS. Subjects came from an ongoing in-hospital study of clinical characteristics of CFS, and were identified from the general population of Wichita, Kansas. We evaluated 145 participants in the study (43 with CFS, 59 with non-syndromic unexplained chronic fatigue, and 43 non-fatigued controls). Multiple SNPs (cut off, minor allelic frequency >10%) were selected by the Applied Biosystems (ABI) SNPbrowser 2.0, and genotyping was done using the validated TaqMan genotyping assay kits from ABI. Genotyping assays used DNA amplified by the GenomiPhi DNA Amplification kit (Amersham Biosciences). Haplotype analysis was done using Haplo.stat software. We observed association ($p<0.05$) of multiple NR3C1 SNPs with CFS when compared to nonfatigued controls. These results were supported when quantitative measures of fatigue were analyzed in all subjects. Evaluation of common haplotypes for association with CFS and quantitative measures of fatigue identified associated haplotypes ($p<0.05$) consistent with the individual SNPs. These data implicate variations in the glucocorticoid receptor gene as a possible mechanism through which the alterations in immune function, HPA axis regulation, and behavior characteristic of CFS may manifest.

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Cholinergic System Genes and Bipolar Disorder. *J. Shi[1], E. Hattori[2], H. Zou[1], J.A. Badner[1], S.L. Christian[1], E.S. Gershon[1], C. Liu[1].* 1) Department of Psychiatry, University of Chicago, Chicago, IL; 2) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute (BSI), Wako, Saitama, Japan.

Bipolar disorder (BP) is a serious mental illness with a life time prevalence of about 1% of the world population. Since the cholinergic dysfunction was proposed for the pathogenesis of BP, we have performed a systematic association study of cholinergic system genes in BP. We have genotyped 58 single nucleotide polymorphisms (SNPs) in 16 genes (CHAT, CHRM1, CHRM4, CHRM5, CHRNA1, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1, CHRNB2, CHRNB3) in two series of samples: the National Institute of Mental Health (NIMH) Genetics Initiative pedigrees with 474 samples from 152 families, and the Clinical Neurogenetics (CNG) pedigrees with 83 samples from 22 multiplex families. Sib-Transmission/Disequilibrium test (TDT) analysis showed nominally significant transmission bias for three SNPs (CHRNA2: rs7017417, p=0.023; CHRNA3: rs514743, p=0.035; rs1948, p=0.019), and Exhaustive Allelic Transmission Disequilibrium test (EATDT) analysis showed a few haplotypes nominally significant transmission in CHRNA7, CHRNA10, CHRNA3, CHRNA5 and CHRNB1, respectively. However, none of these reached gene-wide significance after correction by permutation. Thus, it is unlikely that these cholinergic genes play a major role in the development for bipolar disorder.

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Glucocerebrosidase alterations E326K and T369M in subjects with and without parkinsonism. *M.J. Eblan, K. Hruska, J. Nguyen, J. Walker, E. Sidransky.* NIMH/NHGRI, National Institutes of Health, Bethesda, MD.

Mutations in the gene for glucocerebrosidase (GBA) result in the rare lysosomal storage disorder Gaucher disease. Clinical observations and neuropathologic data suggest an association between mutations in glucocerebrosidase and parkinsonian syndromes. Mutations in GBA have been identified at a greater than expected frequency in several cohorts with synucleinopathies, including Lewy Body Variant Alzheimer Disease (LBVAD), Diffuse Lewy Body Disease (DLB), and classic Parkinson disease (PD), suggesting that aberrant glucocerebrosidase may serve as a risk factor for the development of parkinsonian symptoms. Included among the alleles identified in the subjects with parkinsonism were E326K or T369M. These alterations have been considered polymorphisms, because they are found only in alleles in patients with Gaucher disease that harbor an additional mutation and are also encountered in normal controls. However, some studies suggest that these alterations reduce enzymatic activity. We sequenced GBA in samples from 254 subjects with parkinsonism, and found 5 with the T369M allele (2%) and 12 with E326K (4.7%). To determine if either allele might have a causative role in the development of parkinsonism, we screened for their presence using SNP analysis in 173 adult controls who lacked any signs of parkinsonism on clinical examination. The E326K allele was identified in 7 controls (4%), while the T369M allele was found in only 2 control subjects (1.2%). This frequency was similar to that seen in the subjects with parkinsonism. These results confirm that E326K and T369M are frequently encountered polymorphisms that, unlike other GBA missense mutations, are unlikely to serve as risk factors in the development of parkinsonism.

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Association between the c.66A>G variant in the Methionine Synthase reductase and nonsyndromic cleft palate.

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Cleft palate (CP) is one of the most frequent congenital malformations in humans. It can occur as part of a syndrome, or, most commonly, be non-syndromic (CPO). There is evidence indicating that both genes and environmental factors are implicated in the etiology of CPO. So far very little is known about genetic susceptibility genes and the molecular mechanisms causing the failure of the secondary palate to close. We used a case-parent triad design to examine whether common variants in genes encoding folate metabolism enzymes are risk factors for CPO, and analyzed 64 complete CPO Italian triads. All individuals were genotyped for c.677C>T and c.1298A>C variants in the methylene tetrahydrofolate reductase (MTHFR), c.2756A>G variant in the methionine synthase (MTR) gene, and the c.66A>G methionine synthase reductase (MTRR) gene. Association was assessed using transmission disequilibrium test (TDT) and relative risks of variant alleles in mother or child were calculated by log-linear Poisson regression model. While no association was evident for variants in the MTHFR and MTR genes, a highly significant (p=0.0075) excess of transmission of c.66G allele from both parents was found for the MTRR gene variant. Estimated relative risks of CPO in children with one or two copies of c.66G allele was 3.01 (95%C.I. 1.52-5.95) and 3.54 (95% C.I. 1.26-9.94) respectively. Catalyzing the conversion of vitamin B12-dependent MTR enzyme into its active state, MTRR plays a role of paramount importance for the conversion of homocysteine (Hcy) to methionine. Our results for the first time indicate the c.66A>G variant, that is associated with altered levels of blood Hcy, as a possible genetic risk factor for CPO, and suggest that impairment of Hcy remethylation may be a crucial step in the etiology of cleft palate.

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Genetic association study of ADAM-12 in late onset Alzheimers disease. *L. Jehu, D. Harold, A.R. Morgan, D. Turic, M. Owen, J. Williams.* Cardiff University Dept of Psychological Medicine, University Hospital of Wales, Cardiff CF14 4XN.

ADAM 12 is a member of a family of disintegrin-containing metalloproteases that have been implicated in a variety of diseases including arthritis, cancer and Alzheimer's disease. In this study we are testing the hypothesis that genetic variation within ADAM-12 is associated with late onset Alzheimers disease (LOAD). Polymorphic markers within ADAM-12 were identified both experimentally by DHPLC (20 SNPs, SNP#), and from dbSNP (25 SNPs, DB#). All SNPs were genotyped in DNA pools of 186 LOAD cases and 186 age- and sex-matched controls using a relaxed significance threshold of 0.1. Fourteen displayed a significant difference in allele frequencies between cases and controls at the p0.1 level. The significant SNPs were individually typed in a larger sample of 546 LOAD cases and 546 controls. A significant association with LOAD was detected with two SNPs: the intronic SNP: DB19 and the synonymous coding SNP: SNP8. There was no significant LD between these SNPs (r2=0.031), implying that these two signals are independent. Stratifying the cases based on the presence or absence of an APOE e4 allele had no effect on the data.

As DB19 was the most significant SNP, we used DHPLC on the sequence around this SNP and identified 12 SNPs (Frg#). We also picked out 13 database SNPs from this region (SAT#). These 25 SNPs were typed in our DNA pools, as before. 3 of the 25 were significant at the p0.05 level and have been typed individually in 546 LOAD cases and 546 controls. One SNP: SAT3 remained significant after individual genotyping. Another 5 SNPs were also significant at p0.05 in the DNA pools but are yet to be individually typed.

In conclusion, although this study is work in progress with individual genotyping for some SNPs yet to be done, and for other SNPs to be confirmed by individual genotyping in new samples (500 extra case + 500 extra controls), our results look promising as our data demonstrates an association of ADAM-12 genetic variations with LOAD.

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Association between DJ-1 gene polymorphism and PD. *G. Annesi, P. Tarantino, F. Condino, P. Spadafora, S. Carrideo, F.E. Rocca, I.C. Cirò Candiano, D. Civitelli, F. Annesi.* Inst Neurological Sci, National Research Council, Cosenza, Italy.

Mutations in the DJ-1 gene have recently been shown to cause autosomal recessive Parkinsons disease. To assess whether an 18 bp insertion/deletion variant in the promoter gene of DJ-1 (g.168_185 del) gene is associated with Parkinsons disease (PD). We analysed 290 patients (180 men and 110 women) with sporadic PD and 283 (126 men and 157 women) unrelated healthy subjects from the same geographical area. The median age at the time of the study was 67 years (range 34-86) for PD patients .To type the g168_185del polymorphism, PCR was performed. The alleles were visualized on a 3% agarose gel stained with ethidium bromide. Statistical analysis of allele frequencies was performed by Chisquare test with P<0.05. The frequency of the DJ-1 deletion allele was found statistically different between the PD patients and control population (2=34.026;df=1; p-value <0.001). The frequency of the DJ-1 genotypes was also significantly different between patients and controls (2= 39.890;df=1; p-value<0.001). The presence of the DJ-1 deletion allele polymorphism increased the risk of developing PD (OR 3.07; 95%CI 2.08-4.54). Our findings indicate that the presence of the DJ-1 deletion polymorphism is associated with a higher risk of developing PD. This is in contrast with two previous European finding that did not show an association between this D-1 variant and PD. The contradictory results obtained in the previous studies between DJ-1 promoter polymorphism and PD could be owing to difference in population stratification, small sample size, poorly matched controls or statistical artefacts.

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Apolipoprotein E and Parkinson disease: A case-control study. *M. Alonso[1], M. Lopez[2], J. Guerrero[1], P. Yescas[1], C. Boll[1], A. Rasmussen[1], I. Familiar[1].* 1) Genetics, National Institute Neurology and Neurosurgery, Mexico DF, Mexico DF, Mexico; 2) Sistemas Biologicos, Universidad Autonoma Metropolitana, Mexico DF, Mexico.

Parkinson disease (PD) is a common neurological disorder with an estimated prevalence of about 1% of the general population older than 60 years. There are a number of families in which PD segregates in an unambiguously Mendelian fashion. To date, nine genetic loci have been reported and four pathogenic genes have been identified: alpha-synuclein, parkin, DJ1 and PINK1 in very few patients. PD is a complex disease with combined genetic and environmental causes and many association studies have focused on the identification of genes that causes susceptibility to PD, among these candidate genes is Apolipoprotein E (APOE). The role of APOE in PD has been investigated by several association studies, but results are inconsistent. In the present study, we determined the differences in the APOE genotype and allele distribution among 233 unrelated Mexican PD patients and 388 controls matched by sex and age. In all cases DNA samples were genotyped for the common APOE polymorphisms epsilon 2, 3 and 4. The frequencies of the APOE alleles were compared by x2 test and Fisher's exact test if necessary. The relative risk was estimated through calculations of the odds ratio (OR) with 95% confidential interval (CI) and only values less than 0.05 were considered statistically significant. We found that genotype APOE 4/3 ($p = 0.01$) and APOE-4 allele ($p = 0.07$) increases disease risk. Stratified analysis revealed that APOE-4 allele increases PD risk only in men ($p = 0.032$). This sex difference may be related to environmental exposures that influence the expression of susceptibility genes.

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Functional variants of the cysteinyl leukotriene 1 and 2 receptors are independently associated with atopy in the Tristan da Cunha population. *M.D. Thompson[1,2], V. Capra[3], J. Takasaki[4], A. Slutsky[5], C. Lilly[6], N. Zamel[7], K.A. Siminovitch[8].* 1) Lab Medicine, Banting Inst, Univ Toronto, Toronto, ON, Canada; 2) Department of Pharmacology, University of Toronto; 3) Lab. of Molecular Pharmacology Dept. Pharmacological Sciences University of Milan; 4) Astellas Pharma Inc., Drug Discovery Research, Molecular; 5) St. Michaels Hospital, Toronto, ON; 6) Harvard Medical School, Pulmonary and Critical Care Division Brigham and Women's Hospital, Boston, MA; 7) Department of Medicine, University of Toronto; 8) Departments of Medicine, Immunology and Medical Genetics and Microbiology, University of Toronto, Samuel Lunenfeld and Toronto General Hospital Research Institutes, Toronto, ON.

Atopy is a well-defined immune phenotype that is reported to be a risk factor for asthma. Among the many loci implicated in asthma, only the cysteinyl leukotrienes receptor genes will be discussed here. The cysteinyl leukotriene 1 (CysLT1 at Xq13.2) receptor gene is implicated in asthma because its product is targeted by anti-leukotrienes. The cysteinyl leukotriene 2 (CysLT2 at 13q14) receptor gene has been implicated in atopic asthma by association studies (Thompson et al. 2002; Pillai et al 2004; Fukai et al 2005; Park et al 2005). Our studies focussed on the highly asthmatic Tristan da Cunha population and a smaller cohort from Boston. General linear modeling suggested the independent association of single nucleotide polymorphisms (SNPs) of CysLT1 (p.G300S) and CysLT2 (p.M201V) with atopic asthma. As a result, we decided to compare the functional changes conferred by each SNP. By contrast with the CysLT2 M201V variant, which was less sensitive to leukotriene D4 (LTD4) (Thompson et al 2003), we show that LTD4 acts at the CysLT1 G300s variant with a significantly greater potency. The fact that all double heterozygotes were atopic, consistent with an additive model ($r=.50$; $p<.001$), suggests that functional CysLT1/2 SNPs modify atopy severity on Tristan da Cunha. Due to the founder effect present, we attempted to control for kinship in order to refine the relationship between CysLT1/2 genotypes and atopy/asthma phenotypes in this population.

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Variants in GSTP1 and Emphysema Distribution in the National Emphysema Treatment Trial. *D. DeMeo[1], C. Hersh[1], E. Hoffman[2], R. Lazarus[1], J. Celedon[1], B. Raby[1], F. Sciurba[3], F. Martinez[5], J. Benditt[4], J. Utz[6], J. Reilly[1], E. Silverman[1].* 1) Dept of Medicine, Brigham & Women's Hospital, Boston, MA; 2) University of Iowa, Iowa City, Iowa; 3) University of Pittsburgh, Pittsburg,PA; 4) University of Washington, Seattle, WA; 5) University of Michigan, Ann Arbor, MI; 6) Mayo Clinic, Rochester, MN.

Genetic investigation in chronic obstructive pulmonary disease (COPD) has been plagued by frequent non-replication of genetic associations;phenotypic heterogeneity may be an important contributor. High-resolution CT scans of the chest allow for quantification of emphysema (a COPD subtype) and may enable definition of more precise phenotypes in genetic studies. We hypothesized that the apical and basal distributions of emphysema may be influenced by genetic factors. We evaluated CT scan emphysema phenotypes in 304 individuals enrolled in the genetics ancillary study of the National Emphysema Treatment Trial. The apical and basal percentages of emphysema were calculated using computer generated voxel measures divided by total voxels in the region. 78 single nucleotide polymorphisms(SNPs) in 20 biological and positional candidate genes for COPD were analyzed. In multivariate models adjusted for pack-years, lung function, age, and sex, different subsets of genetic variants were associated with apical versus basal distributions of emphysema. At -950 Hounsfield Units, SNPs in GSTP1(p=0.005), SERPINE2(2 snps,p=0.007,0.01), and TGFB1(3 snps, p=0.01-0.03) were associated with apical emphysema; SNPs in EPHX1(p=0.01) and LTBP4(p=0.02) were associated with basal emphysema. SNPs in GSTP1(p=0.0007),EPHX1(p=0.01-0.04), TGFB1(p=0.03-0.05), and MMP1(0.02) were associated with emphysema distribution (defined as apical minus basal percent emphysema). GSTP1 also demonstrated significant haplotype association. In the associated GSTP1 haplotypes, the functional SNP Ile105Val may contribute to a lower rate of detoxification of cigarette smoke and higher rates of apical emphysema. Thus, apical and basal emphysematous changes in the lung appear to be influenced by different genes. Funding: K08HL072918,R01HL71393,N01HR76102 ,ALA Career Investigator Award.

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The Gln551Arg polymorphism of the *IL4RA* gene is associated with atopic dermatitis in a prospective study. *J. He[1], M. Chan-Yeung[2], AB. Becker[3], H. Dimich-Ward[2], AC. Ferguson[2], J. Manfreda[3], WT. Watson[3], PD. Par~{(&~}[1], AJ. Sandford[1].* 1) The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, St. Paul~{!/~}s Hospital, University of British Columbia; 2) The Occupational and Environmental Lung Diseases Unit, Department of Medicine, UBC; 3) The Section of Allergy and Clinical Immunology, Department of Pediatrics, University of Manitoba.

Background: The interleukin (IL) 4 pathway is involved in the regulation of IgE production associated with atopic diseases. Many coding polymorphisms have been identified in the IL4 receptor alpha chain (IL4RA) and previous association studies have shown conflicting results. We have reported that the *IL4* -589C/T polymorphism was a risk factor for the development of atopy, asthma, and rhinitis at a group of high risk children.

Methods: This prospective study cohort contained 208 white children at high-risk of developing atopy and atopic disorders because at least one first-degree relative had asthma or two first-degree relatives had other allergic diseases. We investigated whether the Gln551Arg polymorphism of the *IL4RA* gene was associated with atopic dermatitis and other related phenotypes such as atopy, allergic rhinitis and asthma.

Results: In 208 white children, the prevalence of atopic dermatitis at 7 years of age was significantly increased in children with the 551Arg allele (16/95 = 16.8%) compared with children without the 551Arg allele (8/113 = 7.1%). The relative risk (RR) for atopic dermatitis for children with the 551Arg allele compared to children without 551Arg allele was 2.4 (95%CI 1.1 - 5.3, p = 0.028). After adjusting for confounding factors such as sex and intervention group, the RR was 2.7 (95%CI 1.1 - 7.1, p = 0.029). No association was found for the Gln551Arg polymorphism of the *IL4RA* gene with asthma and other allergic diseases at 7 years of age. Conclusion: Our data indicate that *IL4RA* polymorphism plays a role in genetic predisposition to the development of atopic dermatitis in children.

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Genes Mediating Lipid Metabolism and Alzheimer Disease Susceptibility. *K.H. Miller[1], R.P. Friedland[2], G.J. Petot[3], H. Kuivaniemi[4], G. Tromp[4], K.A.B. Goddard[1].* 1) Div. Genetic Epidemiology, Case Western Reserve Univ, Cleveland, OH; 2) Laboratory of Neurogeriatrics, Case Western Reserve Univ, Cleveland, OH; 3) Department of Nutrition, Case Western Reserve Univ, Cleveland, OH; 4) Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI.

Genes mediating lipid metabolism are promising candidates in an investigation of Alzheimer Disease (AD) susceptibility as lipids are a critical neuronal structural component and pathways affecting circulating lipids are associated with neuronal health and disease. To test this hypothesis, 18 SNPs selected to maximize the potential for causality were genotyped in 427 cases and controls from the Case Western Reserve University Alzheimer Center for six genes related to neuronal health and/or lipid metabolism: brain-derived neurotrophic factor (BDNF), transthyretin (TTR), tumor necrosis factor alpha (TNF), low-density lipoprotein receptor-related protein (LRP1), apolipoprotein L-3 (APOL3), and sterol O-acyltransferase 1 (SOAT1). In this predominantly Caucasian population of slightly more (57%) women to men and median age of 75.5, AD status was defined using clinical criteria (specifically NINCDS-ADRDA). Data were compiled, inconsistencies and missing values assessed, and statistical tests executed using SAS software. Significant differences were found between cases and controls using a chi square test at markers in two genes: BDNF ($p=0.0261$) and LRP1 ($p=0.0021$). After correcting for multiple testing by the Bonferroni method, the result for LRP1 remained significant. No significant association with disease was found among SNPs genotyped in the remaining genes. Modification of gene effect by diet will be analyzed using food frequency/remote diet recall data from three life stages. Further analyses will explore haplotypic effects, gene-gene interaction, and the role of potential confounders using a generalized linear model. Results from the sub-analysis will demonstrate genetic involvement in AD pathology and if modifications in diet can alter genetic effects, enhancing our understanding of AD and further elucidating potential treatments.

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Follow-up investigation of twelve proposed linkage regions in Multiple Sclerosis. *B.M. Herrera[1], M.Z. Cader[1], D.A. Dyment[1], J. Tzenova[2], M.R. Lincoln[1], S. Orton[1], S. Ramagopalan[1], G.C. Ebers[1].* 1) Multiple Sclerosis Group, WTCHG, Oxford University, Oxford, United Kingdom; 2) Cardiovascular Medicine, WTCHG, Oxford University, Oxford, United Kingdom.

Multiple Sclerosis (MS) is an autoimmune disease with good evidence for a strong genetic component, and a recently described maternal parent-of-origin. So far, the only replicated linkage and association is to the DRB1-15 allele of the HLA region on chromosome 6p21. As with other complex diseases, multiple suggestive linkage signals have been observed. We have recently reported a whole-genome scan in a sample of 552 affected sibling pairs from 442 families with MS; where several regions of suggestive linkage were found. We have now carried out high-density microsatellite genotyping for twelve of the most promising regions (on chromosomes 1p, 1q, 2q, 4q, 5p, 9q, 10p, 11p, 12q, 17q, 18p and 19p) in a novel dataset of 193 families containing affected aunt-uncle/niece-nephew (AUNN) pairs from the CCPGSMS cohort. These families permit examination of parent-of-origin effects when dividing them into likely maternal (n=103) and paternal (n= 93) trait transmission. The results did not confirm linkage in the overall dataset, in subsets defined by maternal or paternal transmission or in the HLA-DR15-positive subset. Additionally, we were able to establish exclusion for locus specific N greater than 1.3 for all the regions studied. These negative data unable to confirm any linkages for locations outside the MHC raise the possibility that the paradigm that has been used to justify genome searches may be incorrect at least in this disease.

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Association analysis of NRXN1 and NRXN3 provides evidence for synaptic dysfunction in autism. *J.A. Duvall[1,2], N. Kono[1], J.L. Stone[1], S.F. Nelson[1], R.M. Cantor[1], D.H. Geschwind[2].* 1) Dept Human Genetics, Univ California at Los Angeles, Los Angeles, CA; 2) Dept Neurology, Univ California at Los Angeles, Los Angeles, CA.

Autism is a neurodevelopmental disorder that is characterized by language difficulties, social deficits, and repetitive, stereotyped behaviors, and which has a significant genetic component. Recently, evidence for involvement of neuroligin genes has been garnered from several sources. We chose to investigate whether genes that are known to interact with neuroligins at the synapse were also associated, to test the broader hypothesis that synaptogenesis is a key dysregulated biological pathway in autism. Here we investigated neurexin 1 (NRXN1) and neurexin 3 (NRXN3), since certain neurexins, a family of cell surface proteins expressed in neurons, interact with neuroligins to promote synaptic maturation. Neurexins 1 and 3 were densely genotyped with 315 and 357 SNPs, respectively, in 224 trios for an average density of 2-4kb. SNPs that were not in Hardy-Weinberg equilibrium ($p<0.01$), SNPs with more than two Mendelian errors, and SNPs in significant linkage disequilibrium with another SNP ($r2>0.9$) were removed from the analysis. The Transmission Disequilibrium Test (TDT) was performed and each gene was tested for an over-representation of SNPs from expected values. SNPs with $p<0.1$ were over-represented in both NRXN1 and NRXN3 ($p=0.0054$ and $p=0.0076$, respectively) and NRXN1 showed an over-representation of SNPs with $p<0.01$ ($p=0.027$). NRXN3 showed this same trend but did not reach a level of significance due to small numbers ($p=0.072$). Preliminary analysis revealed that the most highly associated SNPs within both NRXN1 and NRXN3 clustered within several small regions of each gene. The most significant p values obtained for neurexins 1 and 3 were $p=0.0001$ and $p=0.0016$, respectively. Haplotype analysis provided further support for these associations. While these findings are highly suggestive, confirmation of these disease-associated SNPs in NRXN1 and/or NRXN 3, and other pathway members would provide further evidence that synaptic maturation defects underlie susceptibility to autism.

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Comprehensive analysis of sequence variation in the peptidyl arginine deiminase type IV gene (*PADI4*) suggests a novel intronic variant is associated with rheumatoid arthritis in the North American Caucasian population. *E. Remmers[1], R. Plenge[2], J. Le[1], A. Lee[3], I. Aksentijevich[1], D. Kastner[1], P. Gregersen[3].* 1) Genetics and Genomics Branch, NIAMS, Bethesda, MD; 2) The Broad Institute, Cambridge, MA; 3) The Robert S. Boas Center for Genomics and Human Genetics, North Shore LIJ Institute for Medical Research, Manhasset, NY.

Peptidyl arginine deiminase type IV (*PADI4*) produces citrullinated peptides by converting arginyl residues to citrullyl residues. Auto-antibodies directed against citrullinated peptides are relatively specific for rheumatoid arthritis (RA). Several *PADI4* gene variants are associated with RA in the Japanese population. We sought to determine whether these or other variants of the *PADI4* gene are associated with RA in Caucasians. A set of 22 SNPs that captures virtually all the *PADI4* variation found in a densely spaced set of 69 SNPs was identified by genotyping the 30 HapMap Project CEU trios and analyzing pair-wise LD among the 69 SNPs. The 22 selected SNPs, plus 3 additional *PADI4* SNPs that were associated with disease in Japanese RA patients, were genotyped in 571 independent Caucasian RA patients from the North American Rheumatoid Arthritis Consortium cohort and 750 controls. The SNP most strongly associated with RA in the Japanese study (rs2240340) was weakly associated with disease in this cohort (minor allele frequency (maf) = 0.45 in cases vs 0.40 in controls, p=0.03). However, a nearby SNP (rs2147333) with lower frequency, but in strong LD with rs2240340 (D'=0.99) was strongly associated with disease (maf = 0.11 in cases vs 0.06 in controls, p=0.00006). The association was not detected in rheumatoid factor negative patients (maf = 0.067, p=0.79) and was strongest in patients with a single copy of the HLA shared epitope (maf = 0.13, p=0.000003). This RA-associated SNP is located within the first intron of the *PADI4* gene and the disease-associated variant disrupts a predicted N-Oct3 transcription factor binding site. These data suggest that, similar to the Japanese population, variation of the *PADI4* gene contributes to RA susceptibility; however, a novel, non-coding variant was associated with RA in the Caucasian population.

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Association of single nucleotide polymorphisms (SNPs) with rheumatoid arthritis (RA) in candidate genes at *IDDM5*. *L.J. Gibbons[1], A. Hinks[1], E. Zeggini[2], J. Worthington[1], A. Barton[1], S. Eyre[1], R. Donn[1], W. Thomson[1].* 1) arc Epidemiology Unit, University of Manchester, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom.

Background: Autoimmune diseases may share a common genetic aetiology, as evidenced by the clustering of these diseases in families, the overlap of susceptibility loci, and the consistent involvement of genes at the major histocompatibility locus (MHC). Two microsatellite markers, *D6S311* and *D6S440*, at the type 1 diabetes (T1D) locus, *IDDM5*, have previously been linked and associated with RA.

Aim: To assess association between RA and polymorphisms in candidate genes within the *IDDM5* locus. Selected genes were oestrogen receptor (*ESR1*), vasoactive intestinal peptide (*VIP*), and those encoding UL16-binding proteins (*ULBP1-3*).

Methods: 82 SNPs were selected and genotyped in 359 RA cases and 372 healthy controls by MassARRAY technology (Sequenom). Allele and genotype frequencies were compared between cases and controls by the ^{2} test. Linkage disequilibrium (LD) analysis and haplotype analysis were carried out using HelixTree (Golden Helix Inc.).

Results: Allele frequencies were out of Hardy-Weinberg equilibrium for 8 SNPs, which were excluded from the analysis. 12 *ESR1* SNPs showed association with RA at the 5% significance level, in comparison with 8 expected. Two *ULBP3* SNPs showed significant association with increased risk of RA: the minor allele of rs2009345 was increased in cases compared with controls (OR 1.37, 95% CI 1.09 - 1.73, p = 0.006), as was the minor allele of rs932744 (OR 1.40, 95% CI 1.12 - 1.75, p = 0.002). A 2-marker haplotype formed by these SNPs was associated with RA (p = 0.01). rs2009345 is in intron 1 of *ULBP3*, while rs932744 maps 461bp 5 of the transcription start site. No *VIP* or *ULBP1-2* SNPs were associated with RA.

Conclusion: All associations must be replicated in an independent data set in order to rule out false positives. Of interest is the association with *ULBP3*: ULBP1-3 bind to and activate natural killer cells, stimulating cytotoxicity and cytokine and chemokine secretion.

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Severity of substance use disorder and interaction of dopamine system genes. *M.M. Vanyukov[1,2,3,5], B.S. Maher[1,2,4,5], B. Devlin[1,2,3,5], G.P. Kirillova[1,5], R.E. Tarter[1,3,5], R.E. Ferrell[1,2,5].* 1) Center for Education and Drug Abuse Research (CEDAR), Dept. of Pharmaceutical Sciences; 2) Dept. of Human Genetics; 3) Dept. of Psychiatry; 4) School of Dental Medicine; 5) University of Pittsburgh, Pittsburgh, PA.

The risk for substance use disorders (SUD) is significantly heritable. The dopaminergic system (DS) has been implicated in the etiology of SUD. The genes encoding components of the DS, including the five dopamine receptor genes (DRD1-DRD5), may contribute to the heritability of SUD liability. The dopamine receptors, comprising the D1- and D2-like families (respectively, D1 and D5; and D2, D3 and D4 receptors) are known to act through the opposite effects on adenylate cyclase. It is thus possible that interaction between the two receptor families (D1xD2) is important as a mechanism of SUD risk variation. To test this hypothesis, we conducted 2-way ANOVAs involving the D1- and D2-family genes in adolescent (12-18 years of age) European-American males, using a validated index of the severity of drug involvement (a scale of the Drug Use Screening Inventory-Revised [Tarter & Kirisci, 2001]) as a quantitative trait. All subjects had a DSM-IV diagnosis of substance dependence. The D1xD2 interactions were detected between DRD1 and DRD2, and DRD1 and DRD3 polymorphisms. These interactions may point at potential mechanisms involved in the development of drug dependence and warrant further investigation involving phenotypic mediators of SUD liability.

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Interferon Regulatory Factor 6 (IRF6): a Modifier Gene Predisposing to Isolated Cleft Lip with or without Cleft Palate in the Belgian Population. *M. Ghassibe[1], B. Bayet[2], N. Revencu[1,3], Ch. Verellen-Dumoulin[3], Y. Gillerot[3], R. Vanwijck[2], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology and University of Louvain Medical School, Brussels, Belgium; 2) Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires St Luc, Brussels, Belgium; 3) Center for Human Genetics, Cliniques universitaires St Luc and University of Louvain Medical School, Brussels, Belgium.

Cleft lip with or without cleft palate is the most frequent craniofacial malformation in humans (~ 1/700). Its etiology is multifactorial; some are a result of a genetic mutation, while others may be due to environmental factors, with genetic predisposition playing an important role. The prevalence varies widely between populations and the mode of inheritance remains controversial. The Interferon regulatory factor-6 (IRF6) gene has been shown to harbor mutations in patients with van der Woude syndrome, a dominant form of clefts associated with small pits of the lower lip (Kondo et al. 2002; Ghassibe et al. 2004). Moreover IRF6 has been associated with non-syndromic CL/P in two separate studies (Zucchero et al. 2004; Scapoli et al. 2005). We investigated the role of IRF6 in a set of 195 trios from Belgium. Cleft occurred as an isolated feature. We studied association of the IRF6 locus using two variants: one in the IRF6 gene and the other 100 Kb 3' of the gene. Our independent study group confirms that the IRF6 locus is associated with non-syndromic cleft lip with or without cleft palate. This result, with previous studies performed in the United States and Italy, shows the wide implication of IRF6 in isolated CL/P. It is likely that association to this locus can be identified in various populations and that the IRF6 locus thus represents an important genetic modifier for this multifactorial malformation.
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Investigation of serotonin 1B receptor variants and impulsive aggressive measures in suicide completers. *H. Zouk, G. Csikos, M. Tousignant, M. Séguin, A. Lesage, G. Rouleau, C. Benkelfat, G. Turecki.* McGill Group for Suicide Studies, Douglas Hospital Research Center, Montreal, Canada.

Suicide is a major public health problem that ranks among the top ten causes of death for individuals of all age groups in several countries. Several studies suggest that the predisposition to suicide is the outcome of the interaction of environmental and biological factors. Evidence from family, twin and adoption studies suggests that there is a genetic susceptibility to suicide. Changes in the central serotonin neurotransmission have been described as possible mediators of suicide risk; hence, genes encoding for components of the serotonergic system are promising study targets. One such gene is the 5HT-1B receptor. 5HT-1B knockout mice show greater aggression, anxiety and impulsivity, behaviors that are believe to increase suicide risk. We carried out a study examining this intronless gene located on chromosome 6q14.1. Using SNaPshot multiplex assays, we investigated a total of 2 functional promoter SNPs, 1 SNP in the 3UTR and 2 SNPs in the coding region for their allelic and genotypic frequencies in a sample from the Québec general population consisting of 376 suicide completers and 443 normal controls. We found that the frequencies of the T alleles of the functional promoter 5HT1B -261 SNP (T to G change, $P = 0.016$) as well as that of the 5HT1B 129 SNP (C to T change, $P = 0.001$) are significantly increased among the suicides. Moreover, suicide completers having the T allele of the 129 SNP are significantly more impulsive than those carrying the C allele as determined by the Barratt Impulsivity Scale ($P = 0.039$) and the Novelty Seeking subscale of the Temperament and Character Inventory ($P = 0.007$). Our results suggest that the T allele of the 129 SNP may be associated to the suicide phenotype through the manifestation of higher levels of impulsivity traits.

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The identification of haplotypes in radiosensitive genes associated with risk for adverse skin reactions following radiotherapy in breast cancer patients. *T. Imai[1], T. Suga[1], M. Kohda[1], S. Noda[1], S. Yamada[2], N. Yamamoto[3], A. Ishikawa[1], Y. Ohtsuka[1], H. Suzuki[1], K. Sugawara[1], S. Kawai[1], Y. Michikawa[1], M. Sagara[1], J. Mizoe[2], H. Tsujii[2], M. Iwakawa[1].* 1) Frontier Research Center, National Institute of Radiological Sciences, Chiba, Japan; 2) Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, Chiba, Japan; 3) Chiba Cancer Center, Chiba, Japan.

The goal of radiation therapy in cancer treatment is to achieve a personalized, uncomplicated, loco-regional control. For this, it is of critical importance to have a precise knowledge of the tumoricidal dose for each target tumor and the tolerance doses of normal tissues for each patient. Single nucleotide polymorphisms (SNPs) and derived haplotypes within multiple genes may explain the genetic basis for variations in the adverse reactions after radiotherapy for cancer patients. Using DNA samples collected from 184 Japanese breast cancer patients who qualified for breast-conserving surgery and radiotherapy, we genotyped 905 SNPs from 127 candidate genes for radiation susceptibility. These genes were mainly selected from our gene expression analyses of cultured human cell lines and mouse strains that had exhibited variable radiosensitivities. The SNPs in the candidate genes were selected from jSNP and dbSNP databases and their allele frequencies were examined using 133 healthy controls. The adverse skin reactions were clinically graded according to the National Cancer Institute Common Toxicity Criteria scoring system. Of all 905 SNPs tested, approximately 64% were polymorphic in this cohort of patients. Significant differences were observed in genotype frequencies between the grade 0+1 (n=140) and the grade 2+3 (n=44) groups of skin reactions among the breast cancer patients within 3 months after starting radiotherapy for 24 SNPs in 12 candidate gene loci. Forty-four haplotypes were generated in 7 of the above loci and 4 of these haplotypes were associated with the risk of the adverse skin reactions. These data suggest that the tagged SNPs in the loci may be useful in predicting the risk of adverse reactions following radiation treatment.

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Investigation of TPH2 genetic variants and clinical risk factors for suicide completion in major depression. *C. Lopez de Lara[1], G. Rouleau[1], A. Lesage[1], M. Dumont[1], N. Chawky[1], M. Alda[2], C. Benkelfat[1], G. Turecki[1].* 1) McGill Group for Suicide Studies, Douglas Hospital Research Center, Montreal, Canada; 2) Department of Psychiatry, Dalhousie University, Halifax, Canada.

Suicide is the most serious outcome of major depression, yet not all depressed patients will commit suicide. Several lines of evidence suggest that biological and genetic factors account for at least part of the variability in suicide risk among patients with major depressive disorder. Neurobiological studies have shown overlapping but different neurochemical alterations in suicide and depression, both involving a dysfunction of the central serotonergic neurotransmission characterized by a reduced serotonergic activity. In this context, the gene encoding a second isoform of the tryptophan hydroxylase (TPH2), which is a rate-limiting enzyme expressed predominantly in the brain and involved in the synthesis of serotonin, is an interesting candidate. Here we aimed to identify predisposing factors for suicide taking into account genetic variation at the TPH2 locus while controlling for depressive psychopathology. A total of 14 SNPs in the TPH2 gene and regulatory region, as well as their relationship to several behavioral and clinical risk factors for suicide, were investigated in a sample of 114 suicides that died during an episode of major depressive disorder and 154 depressed controls diagnosed by means of proxy-based interviews. Nine out of the 14 SNPs were intronic, 2 exonic and 3 were located in the regulatory region of this gene. We also carried out linkage disequilibrium and haplotype analyses for these SNPs. We found that three TPH2 SNPs had a significant effect on suicide completion. Moreover, two of these three SNPs, which were located in the regulatory region and in intron 8, had an effect on different TCI (Temperament and Character Inventory) personality dimensions. These results suggest a possible role of genetic variation at the TPH2 gene in suicide completion in major depression as well as a mediating effect through some behavioral traits.

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Association of Y402H variant and clinical subtypes of AMD. *E.A. Postel[1], A. Agarwal[2], P. Gallins[1], S. Schmidt[1], W.K. Scott[1], M.A. Hauser[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration (AMD) is the leading cause of blindness in older Americans, manifested in its most severe forms by neovascular (grade 5) and atrophic (GA, grade 4) changes. Though neovascular disease is responsible for the majority of severe vision loss with AMD, geographic atrophy is also a significant cause of vision loss, and without effective treatment. Therefore, an attempt to clarify its pathogenesis is of utmost importance. Complement Factor H (CFH) is a major AMD risk gene. The Y402H (rs1061170) variant significantly increases the risk for AMD with odds ratios between 2.45 and 5.57, and a population attributable risk of 43%.

To determine if CFH had any effect on determining risk for development of GA in an independent case-control data set of 520 AMD cases and 163 controls, the rs1061170 SNP was tested for association separately by AMD grade. Odds ratios were calculated using standard logistic regression models (SAS version 8.2). There were 407 grade-5 individuals, 107 grade-4, 133 grade-3 (nonatrophic, dry AMD), 35 grade-2, and 128 grade-1 (control) individuals.

There was significant association with AMD when comparing grades 3, 4, and 5 versus the controls. The highest odds ratio was obtained when analyzing the grade-4 cases versus controls (OR = 3.217, P 0.0001). Comparing grade-5 cases versus controls (OR = 2.503, P 0.0001) and grade-3 cases versus controls (OR = 2.143, P = 0.0002) were also highly significant.

In summary, CFH increases the risk of developing GA as well as neovascular and milder AMD. It remains to be seen whether other genes or environmental risk factors such as smoking (Scott et al., this meeting) or diet play a role in determining whether a patient develops exudative or atrophic disease.

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Misregulation of Circadian Clock in Fmr1/Fxr2 Knockout Mice. *J. Zhang[1], Z. Fang[1], K. Kaasik[2], C.C. Lee[2], B.A. Oostra[3], D.L. Nelson[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, USA; 2) Biochemistry and Molecular Biology, University of Texas-Houston Medical School, USA; 3) Clinical Genetics, Erasmus University, The Netherlands.

Fragile X syndrome, the most common heritable cause of mental retardation, is caused by loss-of-function mutations in the fragile X mental retardation (FMR1) gene resulting in the absence of the gene product (FMRP). FMRP belongs to a family of proteins that includes the Fragile X related proteins 1 and 2 (FXRP1 & FXRP2). FXRPs share high similarity in their functional domains with FMRP. It is likely that FXRPs play redundant roles with FMRP. Previous studies have indicated that sleep problems occur in young fragile X patients. Fruit fly *Drosophila* models carrying loss-of- function alleles of dfxr have shown defects in circadian activity. Circadian rhythm abnormalities observed in patients and in *Drosophila* led us to study circadian rhythm in mice with mutations in Fmr1 and Fxr2 gene. We observed abnormalities in circadian controlled locomotor activity in Fmr1, Fxr2 single knockout mice and Fmr1/Fxr2 double knockout mice, with double knockout mice exhibiting a profound defect, comprised of loss of circadian control and hyperactivity. Single knockout animals showed elongated period length in the absence of light cues. There are three possible mechanisms (central clock defect, output pathway defect or input defect) that may attribute to the circadian phenotype in Fmr1 family knockout mice. We examined whether the central clock pathway is intact in the Fxr2 single and the Fmr1/Fxr2 double knockout mice. We tested the expression of 6 central clock components: Clock, Bmal1, Per1, Per2, Cry1 and Npas2. We found that RNA levels of Per1, Cry1 and Npas2 were altered significantly in Fxr2 knockout mice and RNA levels of Per1, Npas2 and Clock were changed dramatically in Fmr1/Fxr2 double knockout mice. In addition, we found that levels of Fxr2 RNA fluctuated with time both *in vivo*(mouse liver) and *in vitro*(mouse embryo fibroblast cell line). These data suggest a role for the Fmr1 gene family in control of the central clock.

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Animal models of Andersen-Tawil syndrome carrying mutations of KCNJ2 recapitulate the human phenotype. *Y. TONG[1], K. ZHANG[1], W. TINGLEY[2], Y.H. FU[1], L. PTACEK[1].* 1) Neurology, UCSF, SF, CA; 2) Gladstone Institute, SF, CA.

Andersen-Tawil syndrome (ATS) is an autosomal dominant disease characterized by cardiac arrhythmias, periodic paralysis and dysmorphic features. Mutations of the KCNJ2 gene, which encodes the inward rectifying potassium channel subunit Kir2.1, have been identified in affected patients. The D71V mutation has the strongest dominant negative effect on Kir2.1 channel function in vitro while R218W is the most frequent mutation found in ATS families. Based on these data, we generated three lines of transgenic mice, WT, D71V and R218W, by bacteriophage--mediated transgenesis. The coding region (including partial 5UTR/3UTR) of mouse KCNJ2 was amplified from a BAC clone (RP23-408D5), and ligated into the pIRES2-EGFP vector. The FRT-neo/kan-FRT fragment from pKD4 plasmid was fused to the C-terminal end of GFP and used as selection marker. Point mutation of D71V or R218W was introduced into the KCNJ2 coding region respectively. The cassette DNA consists of KCNJ2 genomic sequence (WT, D71V or R218W with flanking DNA), IRES-GFP, and FRT-neo/kan-FRT. This was co-transformed with the original BAC clone RP23-408D5 by electroporation. Positive recombinant BAC clones were selected using chloramphenicol-kanamycin. Then, MAMA PCR was performed to identify of WT, D71V and R218W BAC clones. Finally, the FRT-neo/kan-FRT DNA was removed by L-rarbinose in an EL250 bacterial strain. The final BAC constructs (WT, D71V and R218W) were verified by sequencing and injected into mouse oocytes to generate transgenic founders. Southern blot confirmed the transgenic lines. The expression of WT, D71V, and R218W mutations were proved by RT-PCR and sequencing. Preliminary experiments found cardiac arrhythmias and LQT among several mutant transgenic mice. WT-Kir2.1 transgenic mice were resistant to glucose-insulin-induced paralysis while non-transgenic littermate controls developed paralysis. Further work is ongoing to characterize these and other phenotypes in these mice.

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If a virus causes diabetes, what are the implications for genetic predisposition? *W. Klitz[1], B. Niklasson[2].* 1) Sch Public Health, University of California, Berkeley, CA; 2) Apodemus A.B., Stockholm, Sweden.

A variety of evidence points to the role of Ljungan virus (LV), a recently discovered picornavirus, in diabetes and reproductive diseases. LV has been found in the native mammals of Sweden, and governs the population cycles of several native rodents of northern Sweden. LV is also present in North American rodents. In native voles and laboratory mice, LV causes a variety of symptoms mimicking T1D and T2D. Kochs postulates for pathogen transmission have been satisfied in laboratory mice. Antiviral therapy reduces the frequency of diabetes in laboratory mice. In humans the incidence of new cases of T1D, gestational diabetes, preeclampsia and intrauterine fetal death (IUFD) in northern Sweden is correlated with rodent population cycles, showing that the attributable risk due to the underlying factor must be substantial. These diseases are found concurrently in individuals far in excess of random expectations. Antisera and IHC (immunohistochemical) assays show an excess frequency of LV in T1D patients and LV in samples of IUFD from several organs and from placentas of women with preeclampsia. RT-PCR confirms the presence of LV in preeclampsia placenta. In a case control study using the IHC assay LV is positive only in preeclampsia cases. At last, an often posited environmental factor in autoimmunity may be at hand. If LV causes these diseases, then how can genetic predisposition? The diverse genetic associations of T1D, T2D and gestational diabetes suggest that physiological predisposition to specific metabolic processes of each disease must underlie these relationships, rather than reflecting response to a specific pathogen. The variability in response among individuals for disruption of metabolism and in various tissues by LV requires further exploration.

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Large scale mutation analysis of the third glaucoma-causing gene, *WDR36*, at *GLC1G* in the French-Canadian population of Québec. *V. Raymond[1], S. Dubois[1], A. Marquis[1], R. Arseneault[1], J.-L. Anctil[2], A. Duchesne[1], M.-A. Rodrigue[1], The Québec Glaucoma Network.* 1) Ocular Genetics & Genomics, Molecular Endocrinology & Oncology, Laval University Hospital (CHUL) Research Ctr, Québec, PQ, Canada; 2) Ophthalmology Dept, St-Sacrement Hospital, Québec City, PQ.

Glaucoma is a leading cause of blindness worldwide. Of all the different forms, primary open-angle glaucoma (POAG) occurs most frequently and 9 loci have been localized for the disorder. *WDR36* was recently characterized as the third POAG gene mapping to *GLC1G* at 5q22.1 (*Hum Mol Gen* (2005)14:725). To assess the prevalence of *WDR36* mutations in the French-Canadian population, we screened its 23 coding exons for variations in 308 unrelated POAG patients recruited in the Province of Québec, Canada. 107 asymptomatic persons were used as controls. The 23 exons and their intron-exon junctions were screened by direct genomic sequencing. A minimum of 254 POAG subjects with ocular hypertension 21 mm Hg and 15 POAG patients with normal ocular tension were sequenced for each exon. Overall, 23 sequence alterations were detected. 18 of these encoded amino acid (AA) changes while the other 5 were SNPs that did not alter wild-type AA. Of these 18 AA variations, 12 were not identified in controls and were considered potential disease-causing mutations. The T65K (1 patient/306 POAG), D126N (1/298), K284T (1/300), I292T (1/300), N351T (1/303), H411R (1/300), N626T (1/301) and G851R (1/273) potential mutations were novel. The N355S (1/303), A449T (3/295), R529Q (1/295) and M671V (1/309) potential mutations have been described previously. The most common disease-susceptibility mutation observed, H212P (previously reported as Y216P), was detected in 13/296 (4.4%) glaucoma probands and only once (1/106) in controls. The L25P susceptibility mutation was observed in 8/306 POAG probands and 1/107 controls. The D658G alteration was found in 3/309 patients and 3/107 controls. Overall, the frequency of potential disease-causing and potential disease-susceptibility mutations was estimated at 10 % in our unrelated POAG patients. Our data thus demonstrate that *WDR36* mutations are a major cause of glaucoma in the French-Canadian population.

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OPA1 DNA sequence variants in normal tension glaucoma patients. *J. Wiggs[1], S. Wagner[1], E. DelBono[1], J. Haines[2], L. Pasquale[1].* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Glaucoma is characterized by degeneration of the optic nerve. Although the disease is frequently associated with an elevation of intraocular pressure, in 20-30% of cases the intraocular pressure is normal. Identifying genes that contribute to normal tension glaucoma would be a first step toward defining the underlying pathologic mechanisms of optic nerve degeneration. The OPA1 gene codes for a dynamin-related GTPase that contributes to mitochondrial biogenesis and has been implicated in many cases of autosomal dominant optic atrophy, which is also characterized by a spontaneous degeneration of the optic nerve. To evaluate the role of the OPA1 gene in normal tension glaucoma, we screened a population of 80 normal tension glaucoma patients, 130 high tension glaucoma patients and 80 age and ethnically matched controls. Normal tension glaucoma was defined as optic nerve damage with corresponding visual field changes in at least one eye and intraocular pressure less than 22 mmHg. All of the 29 exons and flanking introns of the OPA1 gene were sequenced in normal tension glaucoma patients. Two novel missense changes (Thr95Met, Lys582Arg) and one previously identified missense change (Ser158Asn) were found. The Thr95Met and Lys582Arg variants were not present in the high tension glaucoma patients or controls. Eleven intronic DNA sequence variants were identified, and one of these, IVS 8+4C>T, was found more frequently in normal tension glaucoma patients than controls (28% compared with 9%, $p < .01$). These results indicate that OPA1 gene defects may contribute to normal tension glaucoma and that mitochondrial dysfunction may be one component of this disease.

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Evaluation of MTHFR, MTR and MTRR polymorphisms in patients with glaucoma associated with hyperhomocysteinemia. *D. Figueiredo[1], J. Auguste[1], E. DelBono[1], J.L. Haines[2], J.L. Wiggs[1].* 1) Dept Ophthalmology, Harvard Medical School, MEEI, Boston, MA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Recent studies have indicated that plasma and aqueous humor levels of homocysteine are elevated in patients with a form of open angle glaucoma called pseudoexfoliation glaucoma. This form of glaucoma is associated with abnormalities of blood vessels and the blood aqueous barrier in the eye, as well as a systemic deposition of a characteristic fibrillar material in the stroma of visceral organs. These results suggest that moderate hyperhomocysteinemia may be associated with pseudoexfoliation glaucoma and may contribute to the vascular abnormalities seen in this syndrome. To determine if the elevation of homocysteine in pseudoexfoliation has a genetic etiology, we evaluated the distribution of alleles at two polymorphic sites in the gene for the enzyme methylenetetrahydrofolate reductase (MTHFR), which is a critical component of homocysteine metabolism, as well as polymorphisms in the methionine synthase (MTR), and methionine synthase reductase (MTRR) genes. 84 patients with pseudoexfoliation glaucoma (PXF), 201 patients affected with adult onset primary open angle glaucoma (POAG), and 107 control patients without either glaucoma or PXF were used for this study. POAG was defined as age of diagnosis greater than 35, intraocular pressure >22 mm Hg in both eyes, and glaucomatous optic nerve damage and visual field loss in at least one eye. PXF patients had evidence of characteristic pseudoexfoliative material on the lens capsule or pupillary margin. The distribution of genotypes for the MTHFR polymorphisms C677T, A1298C, the MTR polymorphisms D294N, D919G and MTRR polymorphism S202L did not differ significantly between PXF patients, POAG patients and controls. These results suggest that the allelic variants of the MTHFR, MTR and MTRR genes selected for analysis in this study do not significantly contribute to the disease in this patient population. Other genetic etiologies for hyperhomocysteinemia may be responsible for this phenotype.

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The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. *J. Qu[1,4], R. Li[2], X. Zhou[1], Y. Tong[3], Y. Hu[4], Y. Qian[2], J.Q. Mo[5,6], F. Lu[1], M.X. Guan[2,4,6].* 1) Dept Ophthalmology/Optometry, Wenzhou Medical Col, Wenzhou, Zhejia, China; 2) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; 3) Fujian Medical University, Fuzhou, China; 4) Zhejiang Provincial Key Laboratory of Medical Genetics, School of Life Sciences, Wenzhou Medical College, Wenzhou, Zhejiang , China; 5) Division of Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; 6) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229.

We report here the characterization of a three-generation Chinese family with Lebers hereditary optic neuropathy (LHON). Strikingly, this Chinese family exhibits high penetrance and expressivity of visual impairment. In particular, the average age-of-onset was 13.9 years in this family. 86% of male and 29% female matrilineal relatives in this family developed visual loss with a wide range of severity, ranging from blindness to normal vision. Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the ND4 G11778A mutation and 35 other variants, belonging to the Asian haplogroup D5. The G11778A mutation is present at near homoplasmy in matrilineal relatives of this Chinese family. Of other variants, the novel homoplasmic A4435G mutation absent in 164 Chinese controls is localized at 3 end adjacent to the anticodon, at conventional position 37 (A37), of tRNAMet. The adenine (A37) at this position of tRNAMet is extraordinarily conserved from bacteria to human mitochondria. Indeed, this modified A37 was shown to contribute to the high fidelity of codon-recognition, the structural formation and stabilization of functional tRNAs. Thus, the A4435G mutation may lead to a failure in mitochondrial tRNA metabolism, worsening the mitochondrial dysfunction associated with the primary G11778A mutation. These imply that the novel tRNAMet A4435G mutation has a potential modifier role in increasing the penetrance and expressivity of the primary LHON-associated G11778A mutation in this Chinese family.

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Mitochondrial DNA Content and the *MTND4* Gene Expression in Leber's Hereditary Optic Neuropathy. *M.W. Seong[1], J.Y. Kim[2], H.Y. Ko[1], J.M. Hwang[3], S.S. Park[1].* 1) Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; 2) Central Blood Laboratory Center, Korean Red Cross, Seoul, Korea; 3) Department of Ophthalmology, Seoul National University Bundang Hospital, Seongnam, Korea.

Leber's hereditary optic neuropathy (LHON) is characterized by acute or subacute bilateral visual loss, primarily caused by mitochondrial DNA (mtDNA) point mutation. However the pathogenic mechanism of visual loss is not clearly unraveled. We investigated the pathogenetic mechanism of LHON by the analysis of mtDNA content and the *MTND4* expression. Absolute mtDNA contents were determined in 17 patients, 8 carriers and 47 normal subjects as the copy number ratio of the *MTCYB* gene to nuclear *RNase P* gene using real-time PCR. All patients and carriers had the 11778GA mutation of mtDNA. For real-time PCR assay of mtDNA content, the reference ranges were established by age groups and the reproducibility was assessed by intra-run and inter-run assay. We also quantified the mRNA expression of two mitochondrial genes (*MTND4* harboring 11778GA and *MTCYB*) relative to a nuclear gene (*GAPDH*) in three subject groups. The mean mtDNA contents in patients, carriers and normal subjects were 894.9 (186.9), 848.5 (221.7) and 1148.6 (406.9) copies/cell, respectively. Patients and carriers had significantly lower mtDNA contents than normal subjects (P=0.001 and P=0.048, respectively). The mRNA expression of both *MTND4* and *MTCYB* tended to be lower in patients and carriers than in normal subjects (statistically insignificant), but *MTND4/MTCYB* ratios were similar among three groups. In conclusion, mtDNA content and the *MTND4* expression are decreased in LHON patients and carriers, and it may be caused by mitochondrial depletion. Mitochondrial depletion may be an additional cause of respiratory defect.

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X-Chromosome Inactivation patterns in Females with Non syndromic Cleft Lip and/or Palate. *J.W. Kimani[1], M. Shi[1], L.L. Field[2], M.L. Marazita[3], J.C. Murray[1].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Univ British Columbia, Vancouver, Canada; 3) Center for Craniofacial and Dental Genetics, Univ Pittsburgh, Pittsburgh, PA.

Clefts of the lip and/or palate (CLP) and cleft palate only (CPO) are common congenital anomalies with a complex etiology. Epidemiological studies have found that CLP is more frequent in males than females with a ratio of 2:1 respectively, but approximately 1:1 in CPO. This altered gender ratio for CLP may be explained by sex differences in timing during craniofacial development, but underlying genetic mechanisms remain unidentified. We hypothesized that differences in X chromosome inactivation (XCI) patterns may provide insight into the etiology of CLP and perhaps explain the excess of males affected with CLP. We collected 149 sister pairs (one affected and one unaffected) from various geographic origins (Philippines, India, Spain, Guatemala, USA and China) and extracted DNA from peripheral blood lymphocytes. The samples were classified into 3 phenotypic groups: CL&P-76(13), CLO-60(15) and CPO-13(2); Bracketed numbers indicate pairs that were uninformative for the marker tested, which utilized a trinucleotide repeat in the androgen receptor gene. The XCI ratio was obtained as an average of three experiments, and differences between sister pairs were calculated using the paired t-test. The observed P values were 0.05, 0.65 and 0.99 for CL&P, CLO and CPO respectively. These results indicate an impact of non-random XCI patterns on CL&P. The P values for the CLO and CPO groups were not significant, although this could be attributed to lack of power due to small sample sizes. It is therefore likely that non-random XCI patterns may explain some of the male tendency towards CL&P: X-linked gene expression in males is limited to one X chromosome, whereas females are generally a mosaic of cells expressing genes from either X chromosome. Phenotypic effects that are linked to the X chromosome may therefore manifest readily in males, but are obscured in females due to non-random XCI patterns. Supported by NIH grants R37-DE-08559, R01-DE-09886, R01-DE016148, P50-DE016215.

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Patients with defects in -adrenergic signaling manifest abnormal CFTR activation in the absence of clinical CF.
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Activation of the cystic fibrosis transmembrane conductance regulator (CFTR) by the -adrenergic signaling pathway is thought to play a central role in the development of cystic fibrosis (CF). To test this hypothesis, we have studied CFTR activity in Albrights Hereditary Osteodystrophy (AHO) patients who have a defect in G_s, a key component of the -adrenergic pathway. To examine this pathway in a tissue where CFTR is present, nasal epithelial cells were harvested from 5 AHO patients and 20 normal controls and tested for cyclic AMP (cAMP) levels in response to isoproterenol (ISO), a -agonist. The response to forskolin, a direct stimulator of adenylyl cyclase, was used to normalize among patients and controls. Beta-adrenergic stimulated cAMP production (mean % ISO/forskolin s.d.) was significantly reduced in the AHO patients compared to normal controls (4013 vs. 6014, $p<0.05$). Activation of CFTR by the -adrenergic pathway was examined *in vivo* using two different assays: nasal potential difference measurement (NPD) of respiratory epithelia and sweat rates in the skin. The means of the NPD and the sweat rate for AHO patients were lower than for normals, but both groups displayed wide variance for each test and their ranges partially overlapped. Two AHO patients with CFTR responses outside of the normal range fell within the CF range for both NPD (-5.5, -5.5mV) and sweat rate (0.3, 0.9mg/20min). Yet constitutive CFTR function was normal in both patients based on normal chloride reabsorption following cholinergic stimulation of the sweat glands. Neither patient has clinical features of CF lung disease such as chronic cough or sputum production, and both have normal pulmonary function tests (FEV_1 100% and 102% predicted). Neither patient has evidence of pancreatic disease (normal serum amylase, trypsin and fecal elastase). Thus, identification of AHO patients with reduced CFTR activation due to a defect in the -adrenergic pathway questions the role of this signaling pathway in the pathogenesis of CF.

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Progress toward the generation of a transgenic ovine model for Huntington's Disease (HD). *J.C. Kitchen[1,2], H.J. Waldvogel[2], S.J. Reid[3], C.S. Bawden[4], R.L.M. Faull[2], R.G. Snell[3], M.I. Rees[1,5].* 1) Department of Molecular Medicine and pathology, University of Auckland Medical School, Auckland, New Zealand; 2) Department of Anatomy with Radiology, University of Auckland Medical School, Auckland, New Zealand; 3) The Neurogenetics group, School of Biological Sciences, University of Auckland, Auckland, New Zealand; 4) Molecular Biology Laboratory, Meat and Wool Division, South Australian Research and Development Institute, South Australia; 5) Molecular Neuroscience Group, School of Medicine, University of Wales Swansea, UK.

Huntingtons Disease (HD) is a dominant neurodegenerative disorder caused by an expanded trinucleotide (CAG) repeat in the IT15 gene for huntingtin. We are developing an ovine transgenic model of HD. Our aim is to develop a relatively late onset model of the disease in an organism with a brain structure more closely resembling humans.

We have isolated the ovine HD homologue using a combination of degenerate RT-PCR, 5/3 RACE, and long-range genomic PCR. The ovine cDNA revealed a 9,378bp reading frame creating a 3,126aa protein and exhibiting an 88% peptide homology to human huntingtin. The ovine *HD* gene contains a CAG motif ranging from 10-12 copies, similar to the primate gene and in contrast to the length in rodents.

Two regions divergent in human and ovine huntingtin have been selected for monoclonal antibody generation in order to assess transgene and wild type expression.

Sequence comparison and immunohistochemical distribution of htt, calbindin, substance P and enkephalin in wild type ovine brain tissue will be reported at this meeting. The transgene microinjections will be completed by the time of this presentation and we will be able to report on the progress of the pregnancies. This project will provide an opportunity to trial intervention strategies *in vitro* and *in vivo*.

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Use of real-time quantitative PCR to detect exonic deletions/duplications of *BMPR2* in familial primary pulmonary hypertension. *M.W. Pauciulo, A.P. Batchman, W.C. Nichols.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Familial primary pulmonary hypertension (FPPH) is an autosomal dominant disorder characterized by occlusion and remodeling of the pulmonary arteries which leads to sustained elevation of pulmonary vascular resistance and progressive right heart failure. Using direct sequencing, previous studies have shown that approximately 50% of FPPH patients have coding sequence mutations in the gene for bone morphogenetic protein receptor type II (*BMPR2*). While effective, direct sequencing of a genes coding sequence does not detect other types of heterozygous mutations such as gross gene deletions or duplications. To date, studies using either Southern blot analysis or RT-PCR of *BMPR2* message to identify exonic deletions/duplications have been limited due to cumbersome methodology and inadequate availability of patient RNA samples. The aim of our study was to validate the use of real-time quantitative PCR in identifying *BMPR2* gross gene deletions/duplications in FPPH patients. We examined patients from 2 unrelated PPH families (A and B) recently reported to carry either a heterozygous deletion of exons 4 and 5 (Family A) or a heterozygous duplication of exon 10 (Family B). Following determination of patient DNA concentration using PicoGreen, gene dosage assays were performed on each patient for all 13 exons of *BMPR2*. Absolute quantitation values for each *BMPR2* exon of the FPPH patients were determined using a standard curve and compared to those of control subjects to look for the presence of exonic deletions or duplications. Our results showed both a heterozygous deletion of exons 4 and 5 in Family A and a heterozygous duplication of exon 10 in Family B, thereby replicating the recent report of these two families. This study demonstrates that real-time quantitative PCR is a viable alternative to either Southern blot analysis or RT-PCR for the detection of *BMPR2* exonic deletions/duplications in FPPH. Moreover, this method is significantly less time consuming than Southern blot analysis and obviates the need for patient RNA samples which can be difficult to obtain.

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Novel Splice-site Mutation in the Pre-mRNA Splicing Gene PRPF31 in a Large Chinese Family with Autosomal Dominant Retinitis Pigmentosa. *c. zhao[1,2], S.S. Lu[1,2], A. Alimov[2], X.L. Zhou[2], F.Q. Xiang[2], N.D. Li[1], K.X. Zhao[1], C. Larsson[2].* 1) Molecular Genetics Lab, TianJin Eye Hospital, TianJin, TianJin, China; 2) Department of Molecular Medicine, Karolinska University Hospital, CMM, Stockholm, Sweden.

Purpose: To identify mutations in a four-generation Chinese family with autosomal dominant retinitis pigmentosa (adRP) and to characterize its clinical phenotype. Methods: Ophthalmologic and electrophysiological examinations of 11 patients with RP were performed. A genome-wide linkage screening was conducted in the family. Direct genomic sequencing was used to evaluate all the exons and flanking intron sequences of the candidate gene PRPF31. A restriction assay was used to confirm the mutation status in the family, and to exclude it as a polymorphism in a reference population. Results: The clinical findings were consistent with a relatively severe and diffuse type of RP. By linkage analysis we mapped the disease gene close to RP11 in chromosomal region 19q13.4. A novel single heterozygous base substitution (G>C) was detected at the beginning of intron 8 in the PRPF31 gene whereby the consensus GT dinucleotide of the intron 8 splice donor site was changed to CT. The mutation co-segregated completely with the disease phenotype, but was absent in the unaffected relatives and 100 reference subjects, thus supporting its pathogenic nature in the family. Conclusions: A novel splice site mutation (IVS8+1G>C) in the PRPF31 gene caused retinitis pigmentosa in the four-generation Chinese adRP family studied. The relatively severe clinical findings in the RP patients and the high penetrance observed support the severity of the mutation detected in this family.

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Spectrum of WFS1 mutations in low frequency sensorineural hearing loss and Wolfram syndrome. *M. Lesperance[1], E.D. Snyder[1], C. Nishimura[2], T.A. Sivakumaran[1], J. Toung[1], R.J.H. Smith[2].* 1) Otolaryngology/Head/Neck Surg, Univ Michigan, Ann Arbor, MI; 2) Dept Otolaryngology, Univ Iowa Hosps and Clinics, Iowa City, IA.

Non-syndromic low frequency sensorineural hearing loss (LFSNHL) affects frequencies at or below 2000 Hz. It is typically delayed-onset and progressive without leading to profound deafness. LFSNHL may result from mutations in DIAPH1 (DFNA1) or WFS1 (DFNA6/14/38), the Wolfram syndrome 1 gene. Mutations causing Wolfram syndrome (WS) are recessive and typically inactivating, whereas LFSNHL mutations are dominant and non-inactivating, usually affecting the C-terminal domain. We screened WFS1 in DNA samples from 61 probands with LFSNHL (15 multiplex, 46 simplex) and 9 probands meeting diagnostic criteria for WS. Sequencing results were compared to those reported in the literature and from 110 control DNA samples. 2/9 WS subjects had biallelic WFS1 mutations (1230-1233 delCTCT/W540X or W613X/D211N), and 3/9 had heterozygous variants (R868G, H323R, or A684V). In 4/9, no WFS1 mutations were found. In multiplex LFNSHL families, 6/15 segregated heterozygous deafness alleles. 4 mutations were previously reported and 2 were novel (2203G>T, W678L and 2575G>T, I802S), both affecting residues conserved in rat and mouse. Known LFSNHL mutations L829P and A716T were found in 2 families each. One A716T haplotype shared common SNPs with Dutch Family W. In simplex families, 6/46 had heterozygous WFS1 variants, 3 of which were novel (V624A, R457S and L557F). The 3 others were A602V, found in controls and in psychiatric disease; M518I, reported in psychiatric disease, and K193Q, reported in sporadic LFSNHL.1 simplex case had biallelic GJB2 mutations (235delC/V37I). Subjects with clinical evidence of WS but without WFS1 mutations may have undetected mutations in non-coding regions; or, mutations in WFS2, which has been mapped but not cloned, may be responsible. Our results demonstrate that LFSNHL inherited as a fully penetrant, dominant, nonsyndromic trait is highly predictive of a WFS1 mutation.WFS1 mutations are much less likely when LFSNHL is sporadic, unilateral, or associated with craniofacial malformations.

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Is autosomal recessive deafness associated to oculocutaneous albinism a coincidence syndrome? *K. Lezirovitz[1], F.S. Nicastro[2], E. Pardono[1], R.S. Abreu-Silva[1], A.C. Batissoco[1], I. Neustein[3], M. Spinelli[2], R.C. Mingroni-Netto[1].* 1) Centro de Estudos do Genoma Humano, Genética e Biologia Evolutiva, IB-Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) Divisão de Educação e Reabilitação de Distúrbios da Comunicação, Pontifícia Universidade Católica, São Paulo, Brazil; 3) Depto de Oftalmologia, Hospital Servidor Público Estadual SP, São Paulo, Brazil.

Ziprkowski and Adam (Arch Dermatol. 1964; 89:151-5) reported deafness associated to oculocutaneous albinism (OCA) in two sibships in the same inbred pedigree. A syndrome associating deafness and OCA was suggested [OMIM # 220900]. Deafness and oculocutaenous albinism were also observed by us in a non-consanguineous family: the proband was affected by both diseases, one of his sisters had only OCA and another sister only deafness. Both the proband and his deaf sister were found to be homozygous for the c.35delG mutation in the *GJB2* gene, the most frequent cause of hereditary deafness. Linkage analysis with markers close to the four OCA *loci* excluded linkage to OCA1, OCA2 and OCA3, but homozygosis in markers for OCA4 *locus* (chromosome 5) was observed. Sequencing of the corresponding gene *MATP* revealed the c.1121delT mutation in exon 5, which leads to a stop codon in position 397 (L374*fs*X397). Deafness and albinism in this pedigree were independent autosomal recessive conditions due to mutations in two different genes. It is noteworthy that parents were not consanguineous. We speculate that the putative syndrome reported by Ziprkowski and Adam might have resulted from the co-occurrence of autosomal recessive deafness and albinism in the same pedigree. Financially supported by CEPID/FAPESP and CNPq/PRONEX.

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Novel mutations in the gene encoding otoferlin (OTOF) in Spanish subjects with non-syndromic profound hearing impairment. *I. del Castillo, M. Rodriguez-Ballesteros, M.A. Moreno-Pelayo, M. Villamar, F. Moreno.* Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain.

Inherited hearing impairment (HI) affects about 1 in 2,000 newborns. Mutations in the gene encoding otoferlin (OTOF, on 2p23) are responsible for a subtype of AR prelingual HI (DFNB9), characterized by a profound hearing deficit and features of auditory neuropathy (AN), i.e. absence of auditory brainstem responses but preserved otoacoustic emissions (OAEs). To date, 15 different mutations have been reported in OTOF. Among them, Q829X is highly prevalent among Spanish subjects with prelingual HI. After getting informed consent, we enrolled 443 Spanish unrelated subjects with AR non-syndromic HI (191 multiplex cases and 252 simplex cases, including 6 simplex cases with a diagnosis of AN). DNA was extracted from blood samples, and a specific test for the Q829X mutation was performed. In subjects heterozygous for Q829X, OTOF exons and exon/intron boundaries were sequenced. In patients with AN not carrying Q829X, OTOF mutations were investigated by sequencing. We found 15 cases biallelic for mutations in OTOF. Nine cases (including 2 with previous diagnosis of AN) were homozygous for Q829X. Five cases (including 3 with AN) were compound heterozygotes for Q829X and a novel OTOF mutation. One subject with AN was a compound heterozygote for two novel OTOF mutations. The novel mutant alleles included one insertion (1180-1181insG), three deletions (1236delC, 1601delC, 2684-2685delGG), one indel mutation (2905-2923del19ins11), one nonsense mutation (C883X), and one splice site mutation (IVS35+1G>T). All subjects biallelic for OTOF mutations had a prelingual profound HI. All subjects in our cohort with a previous diagnosis of AN and profound HI were biallelic for OTOF mutations. DFNB9 hearing impairment accounts for at least 3.4 % of cases in our cohort. Our results further support that genetic diagnosis of subjects with AN and profound HI should be directed to the OTOF gene. Our data are of concern to universal screening programmes which use transient-evoked OAEs as the first detection test for HI in newborns, since this technique may overlook a non-negligible proportion of cases.

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Genotype-Phenotype correlations of GJB2 mutations. *K. Kahrizi[1], M. Malekpour[1], C. Nishimura[2], Y. Riazalhosseini[1], A. Daneshi[3], P. Majidpour[3], M. Avenarius[2], R.J.H Smith[2], H. Najmabadi[1].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 3) Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

In this study, we analyzed the genotype-phenotype correlations in the Iranian GJB2-related deaf population. Included in this study were 154 patients who met the defining criteria for autosomal recessive non-syndromic hearing impairment. Hearing loss was non-progressive in all of study patients. Of these, 95 patients (65%) were homozygous for the 35delG mutation and 25 patients (17%) carried the 35delG mutation *in trans* with another GJB2 mutation. The next two most prevalent mutations were W24X, present on 17 chromosomes (5.8%) and -3170G>A, present on 14 chromosomes (4.8%). In the entire group, severe-to-profound deafness was seen in 83.8% of the cases, including 92% of those homozygous for the 35delG mutation. All persons segregating missense mutations, whether homo- or heterozygous, had severe-to-profound hearing loss.

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A *Coch* knock-in mouse model for DFNA9 deafness. *N.G. Robertson[1], T.A. Sivakumaran[1,2], S.A. Hamaker[1], C.C. Morton[1,2].* 1) Depts. Ob/Gyn & Pathology, Brigham & Women's Hospital; 2) Harvard Medical School, Boston, MA.

COCH, encoding cochlin, is expressed at high levels in the inner ear. Five missense mutations in the FCH/LCCL domain of *COCH* have been found in the autosomal dominant late-onset deafness and vestibular disorder, DFNA9, showing abnormal eosinophilic deposits in the cochlear and vestibular labyrinths. To create a mouse model for DFNA9, we generated a mouse knock-in by introducing one of the *COCH* mutations (G88E). A recent *Coch* knock-out mouse does not show any hearing or vestibular defects (Makishima *et al.*, Hum. Genet., in press) supporting a hypothesis of a dominant negative effect of the mutations.

The targeting construct consists of ~5.6kb of 129/SvJae mouse *Coch* genomic sequence spanning introns 3 to 8. Positive and negative selection were performed through neomycin (*neo*) flanked by *loxP* sites in *Coch* intron 5, and thymidine kinase upstream of the *Coch* sequence, respectively. J1 embryonic stem (ES) cells were transfected with the *Coch* mutant construct and 240 ES cell clone were screened. Integrity of the recombination, *loxP* sites, the mutation, and exons were confirmed by PCR, Southern blots, and sequencing. A homologous recombinant ES cell clone was microinjected into C57BL/6 blastocysts for implantation into pseudopregnant mice, yielding several chimera which showed germline transmission of mutant *Coch*.

F1 mice heterozygous for the mutant allele were mated with EIIa-*cre* "deletor" mice for *neo* excision. Prior to *neo* excision, RT/PCR in heterozygotes showed only the wild-type transcript, indicating that the presence of *neo* in the intron disrupts transcription or splicing of mutant *Coch*. These mice are being mated together to generate a *Coch* functional knock-out. After *neo* excision, RT/PCR of knock-in heterozygotes showed presence of the mutant *Coch* transcript. Heterozygotes are being bred for homozygosity. Studies of these mice will include auditory brainstem response (ABR) analysis, vestibular testing, histopathology and immunohistochemistry of all major organs including the inner ear, and cochlear cDNA microarray and proteomic analyses.

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Analysis of *TRPA1* as a Candidate Gene for Bilateral Sensorineural Hearing Loss. *J. McCallum, D. Yaeger, I.D. Krantz.* Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Non-syndromic bilateral sensorineural hearing loss (NSBLSNHL) is genetically heterogeneous with over 80 loci and 30 genes implicated. Approximately 50% of recessive NSBLSNHL is due to mutations in the *GJB2* gene. A recent and growing body of evidence (biophysical, immunochemical, and molecular) suggests that TRPA1 (transient receptor potential ion channel A1; aka ANKTM1) may play a significant role in the signal transduction pathway in hair cells acting as the gated spring on sterocilia tip links in mechanotransduction which results in the perception of sound. *TRPA1* is a 27 exon gene that is defined in its N-terminal region by a series of ankyrin repeats. To assess the contribution that mutations in this gene make towards the etiology of NSBLSNHL we have performed mutational analysis of *TRPA1* in a cohort of 70 probands with NSBLSNHL from a pediatric population. All individuals were evaluated by a clinical geneticist to rule out syndromic etiologies. The cohort did not carry biallelic mutations in *GJB2* and were negative for the A1555G mitochondrial mutation and the *GJB6* deletion. A panel of 80 normal controls (160 chromosomes) was also screened. All exons and intron/exon boundaries of *TRPA1* were amplified by PCR followed by CSGE. All identified shifts were sequenced. Sixteen exonic changes were identified, of which 7 lead to an amino acid change. Two missense changes (each seen heterozygously in a single proband) were not identified amongst the controls: D306N and I469V both of which are found within ankyrin repeat regions. The isoleucine at position 469 is conserved in human and mouse, whereas the aspartic acid at position 306 is conserved in human, mouse, zebra fish and drosophila. The proband with the I469V change has severe to profound hearing loss and no history of affected family members. The proband with the D306N change also has a heterozygous V37I *GJB2* change with mild to moderate hearing loss (this proband is adopted and no parental samples are available). Additional work is underway to characterize these mutations and to better understand the contribution that changes in *TRPA1* make towards the etiology of NSBLSNHL.

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A mutation spectrum of *MYO7A* associated with Usher syndrome type 1 and evidence for the existence of *DFNB2*. *S. Riazuddin[1], S. Nazli[2], R.S. Sheikh[2], S.N. Khan[2], F. Sabir[2], F.T. Javid[2], E.R. Wilcox[1,3], Z.A. Ahmed[1], S. Riazuddin[2], T.B. Friedman[1].* 1) LMG, NIDCD, National Institutes of Health, Rockville, MD; 2) National Center of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan; 3) DNA Sequencing Center, Brigham Young University, Provo, UT, USA.

Defects of myosin VIIa are responsible for splayed and disorganized stereocilia of hair cells of the inner ear of the deaf shaker 1 mouse, the circler phenotype of mariner zebrafish and Usher syndrome type 1 (*USH1B*) and dominantly inherited, progressive hearing loss (*DFNA11*) in humans. We screened for linkage to *MYO7A* markers in a cohort of 557 consanguineous families segregating severe to profound hearing loss as a recessive trait. In 24 of these families, hearing loss was linked to markers for *MYO7A* and affected members of these families had mutations in this gene. Twenty-three of these families have USH1 associated with 14 novel recessive alleles of *MYO7A*. Based on opthalmological and audiological examinations, affected members of just one of the 24 families segregated only profound, congenital hearing loss and affected individuals were found to be homozygous for an in-frame deletion of a single amino acid in the tail of *MYO7A*. This one family is arguably the first convincing clinical case for the existence of *DFNB2*, which is a very rare phenotype associated with recessive alleles of *MYO7A*. In this study we also describe 14 novel recessive mutant alleles of *MYO7A* associated with USH1.

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Molecular basis of deafness in Portuguese familes exhibiting monoallelic or null mutations in exon 2 of GJB2. *H. Teixeira[1], T. Matos[1], C. Correia[1], O. Dias[3], M. Andreia[3], H. Caria[1,2], G. Fialho[1].* 1) Center of Genetics and Molecular Biology, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; 2) College of Health Care, Politechnic Institute of Setúbal, Setúbal, Portugal; 3) Center of ORL, Faculty of Medicine, University of Lisbon, Lisbon, Portugal.

A first characterization of the Portuguese population led us to the conclusion that mutations in GJB2 gene, encoding connexin 26, are a major cause of sensorineural pre-lingual deafness, as described for other populations. Mutation screening of GJB2 coding sequence has however revealed a high proportion of affected individuals carrying only a single mutant allele or no mutation in GJB2. This study aims at contributing for the elucidation of the molecular basis of deafness in those Portuguese families with sensorineural nonsyndromic deafness and exhibiting monoallelic or null disease-causing mutations in exon 2 of GJB2. Exclusion analysis with polymorphic microsatellite markers corresponding to DFNB1 locus was first performed in order to exclude the cases not linked to this locus. Families in which DFNB1-related deafness was confirmed through this analysis were then screened for the two common non-coding, non-complementary DFNB1-causing mutations, the intron 1 splice donor mutation IVS1+1G-A in GJB2 and del(GJB6-D13S1830) in GJB6, before attempting other strategies. The relevance of refining GJB2 screening in order to unequivocally diagnose non-elucidated cases of nonsyndromic deafness will be discussed together with the analysis of the results obtained.

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Linkage mapping of a major familial Ménière disease locus to human chromosome 14. *M.E.S. Bailey[1], Y. Lowe[1], S. Bevan[2], S.A. Carter[1], J. Brooking[2], K.J. Johnson[3], G.A.J. Morrison[4], A.W. Morrison[5].* 1) Division of Molecular Genetics, IBLS, University of Glasgow, Glasgow, U.K; 2) MRC geneservice, Wellcome Trust Genome Campus, Hinxton, Cambridge, U.K; 3) Pharmacogenomics Division, Pfizer Inc., Ann Arbor, Michigan, U.S.A; 4) Dept. of Otolaryngology, Guys and St Thomas Hospitals, London, U.K; 5) Dept. of Otolaryngology, Royal London Hospital, London, U.K. (retired).

Ménière disease (MD) is a relatively common disorder (approx. 1/2000 of the population) of hearing and balance characterised by episodic hearing loss, tinnitus, and rotatory vertigo with nausea and vomiting. MD has mid-life onset and leads to a significant reduction in quality of life. The pathology results from endolymphatic hydrops leading to increased pressure in the sensory compartments of the inner ear and vestibular system. There is strong evidence that the causes of MD are partly genetic and partly environmental. Although mostly sporadic, approx. 7% of cases are familial, a sibling relative risk of 9 has been estimated, and the worldwide distribution is consistent with a founder effect. HLA associations have been reported.

We have used a set of 19 MD families families consistent with autosomal dominant inheritance in a genome scan to map predisposing loci. We have strongly suggestive evidence for linkage of half the families (HLOD 3.4, = 53% of families linked, 95% penetrance) to a single locus in chromosome 14q21-q22. There were no other strongly supported loci. Meiotic recombination analysis of linked families suggests a critical region of 11.6Mbp. Fine mapping of this region is ongoing, but a preliminary analysis indicates that additional informative markers exist and potentially narrow the critical region to <4cM, with a concomitant increase in the HLOD score to 5.0. Analysis of current and former positional candidate genes has ruled out several, including COCH. We hope that identification of the causative gene in the linked families will suggest candidates for the remaining families and illuminate pathways contributing to susceptibility to the more common, sporadic cases of MD.

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V37I is a GJB2 pathogenic mutation with reduced penetrance leading to non-syndromic sensorineural hearing loss. *H. Bruyere[1], C. Huculak[2], T. Nelson[1], B. Casey[1], M. Van Allen[2], F. Kozak[3], S. Langlois[2].* 1) Department of Pathology and Laboratory Medicine, UBC, Canada; 2) Department of Medical Genetics, UBC, Canada; 3) Division of Pediatric Otolaryngology, UBC, Canada.

BACKGROUND More than a hundred GJB2 mutations have been identified in patients with non-syndromic sensorineural hearing loss (SNHL), but the status of some of them is unclear, as they have been reported as both polymorphisms and pathogenic mutations. One such alteration is c.109G>A (V37I), frequently reported in Asian patients and controls. In the culturally diverse SNHL population tested in our lab, the V37I allele is more frequent than the 35delG, 235delC and 167delT alleles, reportedly the most common GJB2 mutations in Caucasian, Asian and Jewish Ashkenazi SNHL patients. Further studies of V37I frequencies in Asian versus Caucasian and patients versus normal hearing controls were deemed necessary for the accurate interpretation of our patients' results. METHODS We reviewed the clinical charts and molecular results of 80 SNHL patients of Chinese and Caucasian descent and compared the results to the frequency of V37I in 200 ethnically matched controls. We compiled the audiograms of individuals with SNHL to determine whether V37I homozygosity was associated with a specific audiologic profile. RESULTS 12 patients of Chinese descent were found to be V37I homozygous and 10 were V37I heterozygotes. In one family, normal hearing parents were also V37I homozygotes. In the 100 Chinese controls, 1 V37I homozygote and 21 heterozygotes were found. The V37I allele was not found in either the 40 Caucasian patients or the Caucasian controls. The difference in V37I homozygote and heterozygote distribution among Chinese patients and controls is statistically significant (Pearson chi squared test: $p<0.00001$). Audiological reports from V37I homozygote patients showed that they present with mild to moderate, high frequency SNHL, in contrast to the mild to severe SNHL observed in the Chinese patients. CONCLUSION V37I is a common allele in the Chinese population with a carrier frequency of 1 in 5, and plays a role in SNHL as an allele responsible for mild to moderate hearing loss with reduced penetrance.

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Predominance of W24X and absence of 35delG mutations in the Baloochi and Sistani deaf population of Iran: a different population. *A. Naghavi[1, 2], C. Nishimura[3], K. Kahrizi[1], Y. Riazalhosseini[1], S. Rigi[4], N. Mahdieh[1], R.J.H. Smith[3], H. Najmabadi[1].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Molecular-Cellular Biology Department, Khatam University, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 4) Zahedan Welfare Organization, Zahedan, Iran.

Hereditary hearing impairment is a heterogeneous disability showing different patterns of inheritance and involving a multitude of different genes. Mutations in the GJB2 gene, especially the 35delG mutation, have been established as a major cause of inherited and sporadic non-syndromic deafness in various ethnic groups. Because population-specific differences are relatively common, in this study we sought to determine the prevalence and spectrum of GJB2 mutations in two isolated ethnic groups: the Baloochi and the Sistani populations of southeastern Iran. Consanguinity and assortative mating are very common in these populations. We analyzed one hundred Baloochi and Sistani families suffering from autosomal recessive non-syndromic hearing impairment. We performed mutation screening of GJB2 using an allele-specific PCR assay to detect the 35delG mutation. The negative or heterozygous cases for the 35delG mutation were screened by denaturing high performance liquid chromatography (DHPLC) and sequencing analysis. Surprisingly, we did not find the 35delG mutation, the most common GJB2 mutation in the white population and in other parts of Iran, in any of the Baloochi or Sistani patients. We identified GJB2 mutant alleles in 18 chromosomes (9%) including R127H, K122I, W24X, 167delT and M93I. Among them, W24X had the highest frequency (10 chromosomes). Based on these data, the hot-spot mutations in the GJB2 gene in the Baloochi and Sistani population with non-syndromic hearing loss may be different from other ethnic groups in Iran. In addition, these results further indicate the existence of an ethnic bias in the distribution of GJB2 mutations in western Asia.

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Contribution of GJB6 large deletion to the hereditary deafness genetic load in the Iranian population. *P. Imanirad[1, 2], M. Avenarius[3], Y. Riazalhosseini[1], C. Nishimura[3], M. Mohseni[1], N. Bazazzadegan[1], K. Kahrizi[1], R.J.H. Smith[3], H. Najmabadi[1].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Molecular-Cellular Biology Department, Khatam University, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA.

Mutations in the gene that encodes the gap-junction protein connexin 26 (GJB2) at the DFNB1 locus on chromosome 13q12 are the major cause of autosomal recessive non-syndromic sensorineural deafness (ARNSD) in many different populations. A second gap-junction gene, GJB6, also localizes to the DFNB1 interval. Interestingly, the encoded protein connexin 30 is expressed in the same inner-ear structures as connexin 26 and both connexins are functionally related. The importance of GJB6 to normal hearing has been confirmed by the identification of a large deletion (()(GJB6-D13S1830)) involving the first two exons and a part of the third exon of GJB6 and a large region of the upstream sequence in persons with ARNSD. Homozygotes for this deletion and compound heterozygotes carrying (GJB6-D13S1830) and a deafness-causing allele variant of GJB2 have severe-to-profound congenital deafness. The aim of this study was to evaluate the contribution made by this deletion to the ARNSD genetic load in the Iranian population. One hundred and fifty four probands with ARNSD from various regions of the country were screened for this mutation using polymerase chain reaction (PCR) primers that amplified the breakpoint junctions of the deletion. Among them, one hundred and sixteen patients were deaf probands with normal GJB2 alleles and the remaining 38 were heterozygote for only one GJB2 mutation. None of patients screened for (GJB6-D13S1830) was shown to carry this deletion, suggesting that this mutation is not a common cause of deafness in Iran.

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Autosomal dominant optic atrophy and sensorineural deafness due to OPA1 R445H mutation. *P. Amati-Bonneau[1], A. Guichet[2], A. Olichon[3], A. Chevrollier[1], F. Viala[4], C. Ayuso[5], S. Odent[6], C. Verny[7], P. Belenguer[3], J.P. Puel[8], C. Hamel[8], Y. Malthièry[1], G. Lenaers[8], P. Reynier[1], D. Bonneau[2].* 1) Service de Biochimie et Biologie Moléculaire,CHU;INSERM U694,Angers, France; 2) Service de Génétique Médicale, CHU Angers, France; 3) Laboratoire de Biologie Cellulaire UMR CNRS 5088, Université Paul Sabatier, Toulouse, France; 4) Service d'ORL, CHU Toulouse, France; 5) Servicio de Genetica, Fundacion Jimenez Diaz, Madrid, Spain; 6) Service de Génétique Médicale, CHU Rennes, France; 7) Département de Neurologie, CHU Angers, France; 8) INSERM U583, Hôpital Saint-Eloi, Montpellier, France.

Autosomal dominant optic atrophy (ADOA, OMIM 165500) generally starts in childhood and is characterized by a progressive decrease in visual acuity, color vision defect in the blue-yellow hues and optic nerve pallor. In most cases, optic atrophy appears as an isolated disorder but ADOA has been reported to be associated with deafness. Mutations in the Optic Atrophy 1 (OPA1) gene, located on chromosome 3q28-q29, have been found in about 47% of patients affected with ADOA. The OPA1 gene encodes a dynamin-related GTPase, located in the mitochondrial inter-membrane space, which plays a key role in controlling the balance of mitochondrial fusion and fission. We report 5 patients from 4 unrelated families affected with optic atrophy associated with sensorineural deafness. The same heterozygous R445H mutation in OPA1 was discovered in all 5 patients. In 3 of the 5 patients, audiological investigations indicated that the deafness was probably due to auditory neuropathy. Hyperfragmentation of the mitochondrial network and decreased mitochondrial membrane potential were observed in the skin fibroblasts of 3 patients. In a complementary study, we investigated the expression profile of OPA1 in the guinea pig cochlear cells. These findings suggest that the occurrence of sensorineural deafness associated with optic atrophy in patients with the R445H mutation could be the consequence of abnormalities of mitochondrial network dynamics and show the importance of a careful examination of auditory performance in ADOA patients.

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Expression of *GJB2* and *GJB6* is reduced in a novel DFNB1 allele. *E. Wilch[1], M. Zhu[2], K. Burkhart[2], M. Regier[3], J. Elfenbein[4], R. Fisher[5], K. Friderici[1,2,5].* 1) Genetics Program; 2) Department of Microbiology & Molecular Genetics; 3) Department of Epidemiology; 4) Department of Audiology & Speech Sciences; 5) Department of Pediatrics & Human Development, Michigan State Univ, East Lansing, MI.

Mutations in *GJB2*, the gene encoding gap junction protein connexin 26 (Cx26), are the most common cause of prelingual-onset, recessively inherited, non-syndromic, sensorineural hearing loss (SNHL) in humans. *GJB2* and *GJB6*, the gene encoding connexin 30 (Cx30), together comprise the DFNB1/A3 locus at 13q12. Although more than 70 mutations of *GJB2* are known or suspected to cause recessive nonsyndromic SNHL, *GJB2/GJB6* mutation screening consistently yields a significant proportion of deaf subjects bearing only one identified mutation, leading to the speculation that unidentified mutations exist at the DFNB1 locus, beyond the proximal promoter region of *GJB2* that is most commonly screened, in *cis*-regulatory elements that control expression of the Cx26 message. We have identified four such *GJB2* heterozygotes in a large kindred of German descent. Each of these profoundly deaf individuals bears a single 35delG mutation, and all share a common haplotype across >250kb on their non-35delG chromosome. We sequenced the entire coding regions and 5 and 3 UTRs of both *GJB2* and *GJB6* in our affected probands, including splice sites, alternative exons and promoter regions, the entire single intron of *GJB2* and >5kb upstream of *GJB2*, and found no sequence variants unique to the novel haplotype. Heterozygosities found in sequencing also showed that both genes are intact on both chromosomes and that affected individuals do not bear the >300kb deletion del(*GJB6*-D13S1830) that segregates with deafness when present in trans with a *GJB2* mutation. Southern blotting indicated no rearrangements or unusual methylation around *GJB2*. However, PCR-based allele-specific expression assays showed that expression of both *GJB2* and *GJB6* from the novel allele is significantly reduced. This is the first evidence of a deafness-associated regulatory mutation of *GJB2*, and the first genetic evidence of potential coregulation of *GJB2* and *GJB6*.

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Knock-in mouse model of Usher type IC and cloning of the Zebrafish *USH1C* ortholog. *J. Lentz[1], S. Sampath[1], S. Ng[1], A. Flynn[1], P. Deininger[2], B. Keats[1].* 1) LSU Health Sciences Center, New Orleans, LA; 2) Tulane University Health Sciences Center, New Orleans, LA.

Usher syndrome type I is characterized by deafness at birth, vestibular dysfunction and progressive retinitis pigmentosa beginning in early adolescence. A cryptic splice site mutation (216GA) in exon 3 of the *USH1C* gene on 11p, which encodes a PDZ-domain protein, harmonin, was found in Acadian Usher type IC families in south Louisiana. In vitro analysis using constructs containing the mutant 216A and subsequent analysis of patient cells lines revealed a 35 base deletion. In order to analyze the impact of this frame-shift mutation, we are creating a knock-in mouse model containing the human 216GA mutation. The targeting construct contains two 4-kb homology arms cloned from mouse DNA, a human fragment with the 216GA mutation cloned from a patient, and the neomycin resistance gene (neo) flanked by loxP sites. Sequence analysis confirmed orientation. Linearized construct was electroporated into embryonic stem (ES) cells and drug (neo) selected for ten days. ES cell clones were analyzed for targeted homologous recombination by polymerase chain reaction (PCR) and Southern blot. Of 485 clones screened by PCR, 50 were positive for homologous recombination (10.3%). Clones confirmed by Southern blot are being microinjected into blastocysts and implanted into pseudo-pregnant mice. Following confirmation of germline transmission of the mutation, breeding will continue to obtain heterozygous and homozygous 216GA offspring. Characterization of hearing and vision defects, as well as expression and developmental studies will be performed. In parallel, we are isolating a full length zebrafish *USH1C* cDNA using sequence information from the zebrafish genome and EST databases, together with RACE experiments. Our transcript has 76% identity with the N-terminus of human and maps to LG25, which shares significant synteny with human chromosome 11p. We will characterize this gene and encoded protein, and determine developmental expression patterns by in situ hybridization analysis. Extending our work on Usher type IC to zebrafish will complement and enhance our ongoing mouse and human studies.

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Molecular characteristics of the *GJB2* gene in Korean patients with nonsyndromic hearing loss. *S.I. Cho[1], M.W. Seong[1], B.Y. Choi[2], S.H. Oh[2], E.Y. Ra[1], S.S. Park[1].* 1) Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; 2) Department of Otolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul, Korea.

Mutations of the *GJB2* gene are the most common cause of nonsyndromic hearing loss of autosomal recessive inheritance in the world. The spectrum of mutation is different to ethnicity and the most frequent mutation is 35delG in Caucasian, 167delT in Ashkenazi Jews, and 235delC in some Asian populations. In this study, we analyzed the *GJB2* gene for mutations in forty-three unrelated Korean patients with nonsyndromic hearing loss and forty-eight normal subjects. Direct sequencing was done for two exons and their flanking regions of the *GJB2* gene and gap PCR for large deletion including the *GJB6* gene [del(GJB6-D13S1830)]. Results showed that two patients were 235delC/427CT compound heterozygote and three patients were heterozygous for 109GA, 235delC, or 427CT without other mutations identified. Any of three mutations was not identified in normal subjects. The allele frequency of 235delC, 427CT, 109GA was 3.5%, 3.5 %, and 1.2% in patients. The large deletion including *GJB6* was not identified. We found a haplotype of two linked polymorphisms frequently: [V27I (79GA) + E114G (341AG)]. In patients, five cases (8.5%) were homozygous and twelve (27.9%) were heterozygous for the haplotype. In normal subjects, two (4.2%) were homozygous, and fourteen (29.2%) were heterozygous. This haplotype has a common origin. Previously the haplotype of these two polymorphisms was suggested as a pathogenic mutation in some reports, but the significance is still unclear. In conclusion, *GJB2* gene is obviously an important cause of the Korean patients with nonsyndromic hearing loss, but its mutation frequency is lower than that of Caucasian and similar with those of Chinese and Japanese.

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Genetic studies of the Iranian deaf population. *H. Najmabadi[1], C. Nishimura[2], K. Kahrizi[1], Y. Riazalhosseini[1], M. Malekpour[1], A. Daneshi[3], M. Farhadi[3], M. Mohseni[1], N. Bazazzadegan[1], A. Naghavi[1], M. Avenarius[2], E. Taherzadeh[1], S. Arzhangi[1], Kh. Javan[1], R.J.H Smith[2].* 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 3) Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

During last six years, probands from 1259 families with hearing loss have been referred to our center for genetic testing. In 13 persons, the following syndromic phenotypes were recognized: Usher, Familial Expansile Osteolysis, Meniere, Bilateral Enlarged Vestibular Aqueduct, Waardenburg, Oto-Palato-Digital, Gorlin, Pendred, Stickler and Kallmann. In the remaining 1245 probands, the diagnosis of non-syndromic deafness was made. Pedigrees were consistent with autosomal recessive inheritance in 1167 probands and autosomal dominant inheritance in ten persons. Three families segregated X-linked deafness. In 65 probands, no other affected relative could be identified - we classified these as simplex cases. So far, GJB2 mutation screening has been completed in 1103 patients with autosomal recessive deafness, initially by completing an allele specific polymerase chain reaction (ASPCR) to detect the 35delG mutation. Persons either negative or heterozygous for this mutation were analyzed by denaturing high performance liquid chromatography (DHPLC) and direct sequencing. We found GJB2-related deafness in 172 of 1103 familial cases (15.6%) and in 4 of 65 simplex cases (6.1%). Identified deafness-causing allele variants included: delE120, 167delT, R184P, 310del14, R32H, 314del14, 35delG, IVS1+1G>A, -3170G>A, R127H, W24X, R143W, E129K, 312del14, M93I, W77R, 8insT, 512insAACG, 510insCGAA, 507insAACG, 329delA, 363delC, Q80L. The last four are novel mutations, which have not been reported in other populations. Selected probands and families negative for deafness at the DFNB1 locus are beginning used to complete a genome-wide linkage analysis. In four of these families, the deafness-causing gene has been localized to specific chromosomal regions.

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Oim Mice: A Model to Assess Thoracic Aortic Integrity, Collagen Content and Collagen Crosslinking During Aging. *B.J. Pfeiffer[1], D.A. Wirth[1], C.L. Franklin[2], F.H. Hsieh[3], R.A. Bank[5], C.L. Phillips[1,4].* 1) Biochemistry; 2) Veterinary Pathobiology; 3) Biological Engineering; 4) Child Health, University of Missouri, Columbia, MO; 5) University of Amsterdam, The Netherlands.

Our initial interest in the *oim* mouse focused on the possible extraskeletal vascular complications associated with aging, which is increasingly important as treatments improve and patients live longer. Recent reports demonstrated increased risk of cardiovascular complications in EDS patients that fail to synthesize pro2(I) chains, the result of COL1A2 gene defects. This and a prior study of an OI patient with a similar gene defect as the *oim* mouse highlight a unique molecular mechanism that may alter cardiovascular properties. Type I collagen, normally a heterotrimer of two 1(I) and one 2(I) collagen chains, is hypothesized to be the major contributor to aortic strength (F_{max}) and stiffness (IEM). To assess the role of 2(I) chains in aortic integrity we used the *oim* mouse, which synthesize only homotrimeric type I collagen, $1(I)_3$. We evaluated thoracic aortas from 3, 8, and 18-month old oim, heterozygote, and wildtype mice for circumferential F_{max}/IEM, histology, 1(I)/2(I)/lysyl oxidase mRNA, collagen content and collagen crosslinking. Circumferential biomechanics of *oim* thoracic aortas exhibited reduced F_{max} and IEM in all age classes. Histological analyses of *oim* descending aortas show reduced collagen staining at each age class, confirmed by hydroxyproline analyses. RT-PCR analysis did not show genotypic differences in 1(I) and 2(I) mRNA expression suggesting that the decreased collagen content is not due to pretranslational mechanisms. Together, these findings suggest the reduced *oim* thoracic aortic integrity correlates with the absence of the 2(I) collagen chains and in part with reduced collagen content. In addition, increased pyridinoline crosslinks per collagen molecule were observed in *oim* aortas for all age classes relative to wildtype with lysyl oxidase mRNA significantly increased in 3-month *oim* aortas relative to age matched wildtype aortas. The role of increased collagen crosslinks is uncertain, but may represent a compensatory mechanism for decreased aortic integrity.

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The carboxy terminus of CFTR interacts with glycolytic enzymes. *M. Lisi, R. Amanchy, A. Pandey, J. Mickle.*
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Cystic fibrosis is a life-limiting lung disease caused by the absence or dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel of the ATP-binding cassette (ABC) transporter family of proteins. To discover accessory proteins that bind CFTR and affect function we created Glutathione-S-Transferase (GST) CFTR fusion constructs for the isolation of interacting proteins from native epithelia. Because CFTR is expressed at relatively low levels in human epithelia, and airway epithelia are not readily accessible, we used opercular epithelia from killifish (*Fundulus heteroclitus*) where CFTR is abundantly expressed and easily accessible. Accordingly, epithelia were isolated, homogenized and the lysate was incubated with GST-kfCFTR C-ter (amino acids 1388-1503). Proteins that bound the C-terminus of CFTR were subsequently eluted and resolved by SDS-PAGE. The gel was silver stained and protein bands that were observed specifically with GST-kfCFTR C-ter, but not GST alone, were excised and subjected to in-gel tryptic digestion; the resulting tryptic digested peptides were analyzed by tandem mass spectrometry using a quadrupole time-of-flight mass spectrometer. As a result, we identified aldolase and enolase as interacting partners of CFTR C-ter. Both of these enzymes are cytosolic components of the glycolytic pathway, a multi-step process that converts glucose to pyruvate with a net yield of ATP. Multi-enzyme complexes facilitate metabolic reactions by allowing reaction intermediates to pass directly from one enzyme to the next without needless diffusion through the cytoplasm. The synergy with CFTR would link this enzyme complex to the plasma membrane where glucose import occurs. Moreover, an association of the glycolytic complex with the carboxy terminus of CFTR would bring ATP in close proximity to the nucleotide binding folds for the immediate binding and hydrolysis needed to gate the CFTR chloride channel.

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A new look of disease genes revealed by a comparison with housekeeping genes. *Z. Tu, L. Wang, M. Xu, X. Zhou, T. Chen, F. Sun.* Molecular and Computational Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles, CA.

The Online Mendelian Inheritance in Man (OMIM) has collected more than 2,000 genes that contribute to hereditary diseases. Previous studies compared them with all other human genes to find the common features of disease genes. However, this approach ignores the fact that the human genome also contains a large number of essential genes (genes that are essential for survival and producing progeny). The relationship among essential genes, disease genes and all other genes haven't been systematically studied yet. We hypothesize that human genes form a continuum. In one extreme are those functionally most important genes, the mutations of which at certain residues will severely affect human health and lead to early death. In the middle are those genes when mutated will cause diseases but patients with such diseases can still survive long enough to have progenies. In the other extreme are genes whose mutations generally will not cause detectable pathological phenotypes. In the absence of a set of well defined human essential genes, we choose 1,789 ubiquitously expressed human genes (UEHGs), also known as housekeeping genes, as category I genes and the disease genes in OMIM as category II genes. All other human genes are referred as category III genes. We show by several means that the category I genes are most likely to be essential genes. For example, a mapping of human genes to *C.elegans* genome reveals that category I genes have significantly more homologs which are essential genes in *C.elegans* (41%) than category II genes do (22%, p-value is 1.8e-11). We also show that the three classes of genes have distinct features such as evolutionary conservation, DNA coding length, gene function, etc. Our findings indicate that disease genes have an intermediate essentiality between category I and category III genes. We believe it is more reasonable to divide human genome into the above three classes in the systematic studies on disease genes. These findings and the features we studied show a new picture of disease genes and thus will help people better identify new disease genes.

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A novel locus for primary ciliary dyskinesia (PCD) with inner and outer dynein arm defects in the Pakistani population. *H. Mitchison[1], V.H. Castleman[1], C. OCallaghan[2], E. Moya[3], J.E. Dankert-Roelse[4], G. Pals[5], R.M. Gardiner[1], E.M.K. Chung[1], R. Chodhari[1].* 1) Paediatrics and Child Health, Royal Free and University College Medical School, Rayne Building, London, UK; 2) Division of Child Health, Department of Infection, Immunity, and Inflammation, University of Leicester, UK; 3) Department of Paediatrics, Bradford Royal Infirmary Hospital, Bradford, UK; 4) Department of Pediatrics, University Hospital, Academisch Ziekenhuis van de Vrije Universiteit, Amsterdam, Netherlands; 5) Department of Human Genetics, VU Medical Center, Amsterdam, The Netherlands.

PCD is a genetically heterogeneous autosomal recessive disorder affecting 1:20,000 live births which is characterised by respiratory tract infections, sinusitis, bronchiectasis and subfertility. This arises from cilia dysmotility, associated with various ultrastructural abnormalities. Half of patients exhibit laterality defects (Kartagener syndrome), thought to result from embryonic nodal cilia defects. We have studied 5 consanguineous families with a ciliary defect of absent inner and outer dynein arms from the N. Pakistan Mirpur region. There are 9 affected and 29 unaffected individuals and a 400 marker microsatellite genome wide scan using GENEHUNTER identified a region of statistically significant linkage in 3/5 families, on chromosome 17q21-22. Haplotypes showed a shared region of excess homozygosity across a 9 Mb region between D17S951 and D17S1865. To isolate the PCD gene at this new locus, additional genotyping is in progress to refine the critical region and identify allelic association. We detect 107 gene-coding elements in the region (NCBI Build 35) which vary in their degree of biological confirmation. Vertebrate cilia are highly evolutionarily conserved for structure/function, so using a comparative bioinformatic approach incorporating ciliated and non-ciliated organism genomic and proteomic data, we have prioritised positional candidate genes for mutational analysis according to putative function, expression pattern and conservation in ciliates. This has highlighted appropriately expressed genes in the region for further analysis, including a well studied sperm-associated protein, SPAG9.

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Ehlers-Danlos syndrome classic type: COL5A1 haploinsufficiency disrupts multiple steps in collagen fibril assembly. *R. Wenstrup[1], J. Florer[1], J. Davidson[2], C. Phillips[3], B. Pfeiffer[3], D. Menezes[4], I. Chervoneva[5], D. Birk[4].* 1) Div of Human Genetics, Children's Hosp Research Fndn, Cincinnati, OH; 2) Department of Laboratory Medicine, Vanderbilt University School of Medicine, Nashville TN; 3) Dept of Biochemistry, University of Missouri-Columbia, Columbia MO; 4) Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philidelphia PA; 5) Department of Medicine, Thomas Jefferson University, Philidelphia PA.

The most commonly identified mutations causing Ehlers-Danlos syndrome (EDS) classic type result in haploinsufficiency of COL5A1 which encodes pro 1(V) chains of type V collagen, a quantitatively minor collagen that co-assembles with type I collagen as heterotypic fibrils. Mice heterozygous for a targeted inactivating mutation in col5a1 that caused 50% reduction in col5a1 mRNA and collagen V had, comparable to EDS patients, decreased aortic tensile strength and hyperextensible skin with decreased tensile strength of both normal and wounded skin. In dermis of col5a1 +/- animals, 50% fewer fibrils were assembled with two subpopulations: normal fibrils with periodic immuno-reactivity for collagen V for which type I/V interactions regulate nucleation of fibril assembly; and highly irregular fibrils lacking collagen V, generated by unregulated sequestration of type I collagen. Fusions of normal and aberrant fibril subpopulations disrupt the normal linear and lateral growth mediated by fibril fusion. Therefore, reduced fibril nucleation and dysfunctional fibril growth with potential disruption of cell directed fibril organization leads to the connective tissue dysfunction associated with EDS.

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Consortium for Osteogenesis Imperfecta Mutations: Lethal Regions in the Helical Portion of Type I Collagen Chains Align with Collagen Binding Sites for Integrin and Proteoglycans. *J. Marini[1], A. Forlino[1], W. Cabral[1], A. Barnes[1], J. San Antonio[2], S. Milgrom[1], J. Hyland[3], J. Korkko[3], D. Prockop[3], A. DePaepe[4], P. Coucke[4], F. Glorieux[5], P. Roughley[5], A. Lund[6], K. Kuurila[7], D. Cohn[8], D. Krakow[8], M. Mottes[1], R. Dalgleish[1], P. Byers[9].* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Thomas Jefferson Univ, Phila, PA; 3) Tulane Univ, New Orleans, LA; 4) Univ Ghent, Belgium; 5) Shriners Hospital, Montreal, Canada; 6) Rigshospitalet, Copenhagen, Denmark; 7) Vaasa Hosp, Finland; 8) Cedars-Sinai, Los Angeles, CA; 9) Univ of Washington, Seattle, WA.

To explore genotype-phenotype relationships between mutations in type I collagen genes (COL1A1 and COL1A2, encoding 1(I) and 2(I) chains, respectively) and Osteogenesis Imperfecta, we identified 832 independent mutations, of which 682 result in substitution for glycine residues in the helical domain and 150 alter splice sites. Distinct genotype-phenotype relationships emerge for each chain. One-third of 1(I) glycine substitutions are lethal, especially residues with a charged or branched side chain. Substitutions in the first 200 residues are non-lethal and have variable effect thereafter, unrelated to folding or helix stability domains. Two exclusively lethal regions (691-823 and 910-964) align with Major Ligand Binding Regions, suggesting crucial interactions of collagen monomers or fibrils with integrins, MMPs, fibronectin and COMP. Mutations in COL1A2 are predominantly non-lethal (80%). Lethal substitutions are located in 8 regularly spaced clusters along the chain, supporting a regional model. The lethal regions align with proteoglycan binding sites along the fibril, suggesting a role in fibril-matrix interactions. Unlike 1(I), recurrences at the same site in 2(I) are generally concordant for outcome. Splice site mutations in COL1A1 are rarely lethal; they often lead to frameshifts and the mild OI type I phenotype. In 2(I), lethal exon skipping events are located in the carboxyl half of the chain. These genotype-phenotype relationships indicate that the two collagen chains play very different roles in matrix integrity and that phenotype depends on intracellular and extracellular effects.

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A new locus for an autosomal recessive congenital mucular dystrophy with hyperlaxity. *M. Tetreault[1,2], A. Duquette[1], I. Thiffault[1,2], J. Jarry[1,2], L. Loisel[1], M. Vanasse[3], Y. Robitaille[3], B. Brais[1,2].* 1) Laboratoire Neurogenetique, Hopital Notre-Dame-CHUM, Montreal, Canada; 2) Centre for the Study of Brain Disease, Montreal, Canada; 3) Hopital Ste-Justine, Montreal, Canada.

Joint hyperlaxity and concomitant joint contractures are often observed in different congenital muscular dystrophies (CMD). We have recruited a group of 16 French-Canadian patients from Southwestern Quebec belonging to 13 different families displaying a recessive congenital muscular dystrophy with joint hyperlaxity (CMDH). We have excluded by linkage and sequencing analyses that this new form of CMD is caused by mutations in one of the three subunits of collagen VI mutated in Ullrich CMD, which has an overlapping phenotype with CMDH. We have carried out a genome wide scan (GWS) by genotyping 500 markers at 8cM intervals for 2 families. The GWS results combined to fine mapping analyses demonstrated linkage of all our families to a 10cM chromosomal region not previously known to be linked to a recessive congenital muscular dystrophy (multipoint LOD score 5.3). Haplotypes for markers in the region suggested that the two more common mutations present in the French-Canadian population explain 81% of carrier chromosomes in this population. The identification of the mutated gene in CMDH should shed light on the biological basis of joint hyperlaxity and congenital muscular dystrophy.

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Ocular manifestation of collagen V mutations. *E. Heon[1,3], F. Segev[1,2], W.G. Cole[3,4], R.J. Wenstrup[5], F. Young[3], A.R. Slomovic[2], D.S. Rootman[1,2], D. Whitaker Menezes[6], I. Chervoneva[6], D.E. Birk[6].* 1) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Ophthalmology, Toronto Western Hospital, Toronto, ON, Canada; 3) The Genetics and Genomic Biology Program, The Hospital for Sick Children Research Institute; 4) Division of Orthopedic Surgery, Dept of Surgery, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 5) Division of Human Genetics, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 6) Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University, Philadelphia, PA.

Type V collagen forms heterotypic fibrils with type I collagen. This study aimed to define the ocular phenotype of mutations in type V collagen genes, COL5A1 and COL5A2, and to study the phenotype in a col5a1 deficient mouse.Seven patients with classical Ehlers Danlos syndrome (EDS) due to COL5A1 haploinsufficiency and one with an exon skipping mutation in COL5A2 underwent an ocular examination, corneal topography, pachymetry and specular microscopy. A col5a1 haploinsufficient mouse model of classical EDS was used for biochemical and immunochemical analyses of corneas. Light and electron microscopy were utilized to quantify stromal thickness as well as diameter, density and collagen fibril shape. Five males and three females (mean age: 2613.57yrs; range: 11-52yrs) were studied. All patients had floppy eyelids. Cornea were thinner when compared with controls. In the heterozygous col5a1 mouse, corneal type V collagen content was reduced by 40-60percent, while corneal stroma thickness was reduced by ~26percent. Throughout the corneal stroma, collagen fibril diameters were increased, while fibril density at all developmental stages was decreased. In the eye, COL5A1 and COL5A2 mutations manifest as abnormally thin and steep corneas with floppy eyelids. Mechanisms involved here likely involve heterotypic type I/V collagen fibrillogenesis abnormalities similar to those observed in the col5a1 haploinsufficient mouse cornea. This abnormal corneal thickness likely affects interpretation of intraocular pressure measurements.

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Mutations responsible for Larsen syndrome cluster in the FLNB protein. *D. Zhang[1], J.A. Herring[2], S.S. Swaney[1], T.B. McClendon[1], X. Gao[1], R.H. Browne[1], K. Rathjen[2], C.E. Johnston[2], S. Harris[3], N.M. Cain[1], C.A. Wise[1].* 1) Seay Center for Musculoskeletal Research, TX Scottish Rite Hosp, Dallas, TX; 2) Orthopaedics Dept., TX Scottish Rite Hosp, Dallas, TX; 3) Clinical Laboratory, TX Scottish Rite Hosp, Dallas, TX.

Larsen syndrome is a rare disorder of craniofacial and musculoskeletal systems. Distinct facial features include hypertelorism, prominent forehead, and depressed nasal bridge. Clinical management can be difficult due to extensive skeletal involvement including joints, feet, and spine. Both sporadic and dominantly inherited cases have been described. Mutations in the gene encoding filamin B were recently described in Larsen syndrome. Filamin B is a member of the filamin family of cytoskeletal proteins that induce non-muscle actin polymerization and participate in signal transduction pathways. Filamin B contains 2,602 amino acids that are organized into an N-terminal actin-binding domain (ABD) followed by twenty-four repeating units. It is expressed in chondrocytes throughout the epiphyseal growth plate and may function in chondrogenesis. To further characterize the spectrum of mutations causing Larsen syndrome, and to test genetic heterogeneity, we screened coding exons of the filamin B gene (FLNB) in our collection of sporadic and inherited cases. A single heterozygous missense mutation was found in each case. These mutations affect amino acids that are orthologously conserved in filamin B protein of vertebrate species, and homologously conserved in other filamin family members. All were apparently private, except for a dominantly inherited 679G-A change that was previously reported in a sporadic case. Compilation of our results and other reported mutations in Larsen syndrome revealed a clustered pattern in exons encoding either the ABD or repeat domains 14 or 15 of the filamin B protein. These results suggest that Larsen syndrome is genetically homogeneous and may be entirely explained by mutations in FLNB. Clustered mutations highlight filamin B domains that mediate cellular functions important in early skeletal development. This mutation pattern also may simplify diagnostic and genetic testing for the disorder.

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Skeletal dysplasia and infertility mutations on mouse Chr 4 and 9. *S.M. Faust[1], I.J. Karolyi[1], A. Bartke[2], K.B. Cha[1], A. Hunt[1], K.D. Hankensen[3], J. Moran[4], D.R Beier[4], S.A. Camper[1].* 1) Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Physiology, Southern Illinois University, Springfield, IL; 3) Department of Orthopaedic Surgery, University of Michigan, Ann Arbor, MI; 4) Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Skeletal dysplasias are characterized by abnormal growth of cartilage and bone, and occur in approximately 3.2 per 10,000 births. The gravity of the defects range from asymptomatic minor skeletal abnormalities to major, fatal defects. We report genetic mapping and characterization of two spontaneously arising, recessive mouse mutants with skeletal defects and infertility. Peewee (*pwe*) mutants exhibit disproportionate dwarfism, apparently due to disorganized chondrocytes and ossification without vascularization. Females are infertile, and males are subfertile. *Pwe* maps on mouse Chr 4 near *Mmp16*, a member of the membrane-bound matrix metalloproteinase gene family. *MMP16* maps to human chromosome 8, while neighboring genes of *MMP16* on mouse Chr4 map to a region homologous to chromosome 9 in humans. *MMP14* deficiency causes decreased chondrocyte proliferation, defective vascular invasion of cartilage, and dwarfism, suggesting that *Mmp16* is an excellent candidate gene. Identification of the molecular defect in *pwe* mice could uncover the molecular basis for a human skeletal defect. Chagun (*cha*) mutants are chondrocyte deficient, and males are hypogonadal and infertile. We report refinement of the Chagun (*cha*) locus on mouse Chr 9 in a high-resolution intercross, suggesting that *AK079355* is the responsible gene; virtually nothing is known about its function. This gene corresponds to a region homologous to human chromosome 3. Numerous human skeletal dysplasias have been described but not mapped. Identification of the responsible mutations in *cha* and *pwe* mice may identify unmapped human skeletal dysplasia genes.

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Skeletal Dysplasia in Trps1 mutant mice demonstrates Trps1 repression of Runx2 in the pathogenesis of Tricho-Rhino-Phalangeal Syndrome. *D. Napierala[1], K. Sam[2], Q. Zheng[2], G. Zhou[2], T. Bertin[2], R. Shivdasani[3], B. Lee[1,2].* 1) Howard Hughes Medical Institute; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

Tricho-rhino-phalangeal syndrome (TRPS) is a dominantly inherited craniofacial and skeletal dysplasia characterized by short stature, hip abnormalities, cone-shaped epiphyses, premature closure of growth plates and distinctive craniofacial appearance. TRPS is caused by mutations of the TRPS1 gene located at 8q23-24 and coding for the transcriptional repressor TRPS1. To elucidate the role of Trps1 in skeletogenesis, we analyzed Trps1 mutant mice deleted for the GATA-type DNA-binding domain. Heterozygous mutant mice have normal appearance except of the craniofacial abnormalities. Homozygous Trps1 mutant mice die shortly after birth. Skeletal preparations of newborn homozygous mutant mice demonstrated delayed ossification of vertebrae and sternum, and significantly reduced maxilla and mandible. Histological analysis of growth plates showed the elongation of prehypertrophic and hypertrophic chondrocytes zone and delay in endochondral ossification. The quantitative real-time PCR on mRNA isolated from humerus of 4 week old mice showed dramatic increase of expression of chondrogenic markers - col10a1 and col2a1 as well as elevated expression of Runx2 in heterozygous mice. Runx2 is a key transcription factor involved in osteoblast differentiation and chondrocyte maturation. To test the potential interaction of Trps1 and Runx2, we performed cotransfection studies. In COS7 cells, Trps1 strongly represses Runx2 transactivation of the target 6xOSE reporter, which responds only to Runx2 transactivation. Moreover, the bone-related promoter of Runx2 contains several potential GATA consensus sequences. In vitro analyses showed that Trps1 forms complexes with each of these elements in COS7 but not in ROS17 cell lines. Our data suggest that Trps1 regulates the cartilage to bone transition during endochondral ossification in part by inhibition of Runx2 transcription as well as Runx2 protein function.

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FISH analysis in the molecular diagnosis of Cleidocranial Dysplasia in patients without mutations of *RUNX2*. *K. Sam[1], R. Mendoza-Londono[1], S.A. Yatsenko[1], D. Napierala[2], P. Stankiewicz[1], B. Lee[1,2].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine.

Cleidocranial dysplasia (CCD) is an autosomal dominant skeletal dysplasia with high penetrance and variable expressivity. Clinically, CCD manifests with short stature, delayed fontanel closure, hypoplastic clavicles, and dental anomalies. Most cases of CCD are caused by haploinsufficiency of *RUNX2*, which encodes for a transcription factor essential for osteoblast differentiation and chondrocyte maturation. The majority of mutations described to date are missense mutations located in the conserved RUNT domain, which disrupt RUNX2 binding to the target OSE2 element in osteoblast-specific genes. Sequencing of the coding region of *RUNX2* identifies mutations only in a fraction of individuals with clinical diagnosis of CCD. Microdeletions involving *RUNX2* may account for some of the additional cases. In order to optimize the detection in these individuals we established a combined approach of gene sequencing and fluorescent in situ hybridization (FISH). We studied 40 samples from individuals with clinical diagnosis of classic CCD or CCD-like phenotype. Mutations were identified in 18 (45%) patients, and sequence variants in the *RUNX2* promoter in five(12%) patients. In vitro analyses suggest that some of these promoter variants may affect transcriptional activity of RUNX2 and therefore modify the CCD phenotype. FISH analysis with BAC probes spanning *RUNX2* was performed on 14 of the 22 samples that were negative for mutation. We detected a deletion of one copy of *RUNX2* in three(8%) patients, which corresponds to 14% of the mutation-negative samples. In addition, FISH analysis allowed us to fine-map the breakpoints of a complex translocation, involving 6p21, in a patient with CCD-plus phenotype. Interestingly this patient has a breakpoint 34 Kb upstream of *RUNX2* that may affect a regulatory region, or result in positional dysregulation of the gene. Our results indicate that patients with CCD or CCD-like phenotypes with no mutation in *RUNX2* should undergo FISH analysis.

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High-Throughput Analysis of Candidate Extracellular Matrix Genes in Connective Tissue Disorders. *B. Tinkle[1], W. Cole[2], R. Wenstrup[1].* 1) Div Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Hospital for Sick Children, Dept of Genetics, Toronto, Ontario, Canada.

We have developed a microarray based genetic screening method designed to discover new genetic causation(s) for heritable connective tissue disorders (CTDs) that does not depend on family studies or linkage analysis. For several CTDs, linkage studies have not proved successful because of genetic heterogeneity, small pedigree size, decreased penetrance, variable expressivity, or high prevalence of singleton cases. Our approach takes advantage of prior observations that many CTDs are due to mutations that, through a variety of molecular mechanisms, result in the functional loss of one allele (i.e. haploinsufficiency). Specifically, we have developed an automated Illumina^{TM}-based platform that compares allelic representation of single-nucleotide polymorphisms (SNPs) within exons in genomic DNA with allelic representation in cDNAs generated from total cellular RNA of fibroblasts. A minimum of 3 SNPs per gene with a cumulative frequency >50% are used to optimize informativeness and reduce false positives. Computational algorithms determine if the alleles are expressed approximately equal or below a threshold established to be consistent with haploinsufficiency. A second, parallel set of cDNAs are made from fibroblasts incubated under conditions that block nonsense-mediated decay to restore equimolar expression of the two alleles as a confirmatory step. As proof of principle, we analyzed 14 cell lines across a SNP microarray containing 384 loci in 63 extracellular matrix genes. Eight cell lines from patients with Ehlers-Danlos syndrome were analyzed, 5 of which had been previously shown to have haploinsufficiency of COL5A1 and the remaining 3 were informative but not haploinsufficient. Differential allelic expression was conclusively shown in all 5 lines while no COL5A1 haploinsufficiency could be detected in any of the others. This methodology will be useful for determining disease candidate genes for some CTDs not approachable through traditional genetic methods and may speed the discovery of disease-causing mutations for improvements in diagnostics and therapeutics.

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Genetic Background in Determining Phenotypic Severity in the Osteogenesis Imperfecta Mouse Model. *SM. Carleton[1], DJ. McBride[2], WL. Carson[1], CT. Winkelmann[1], CE. Buff[1], JS. Morris[1], CL. Phillips[1].* 1) University of Missouri, Columbia, MO; 2) University of Maryland, Baltimore, MD.

Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous disease characterized by impaired biomechanical properties of bone. More than 800 type I collagen mutations have been shown to cause OI, with most limited to single individuals or families. Patients with identical mutations may exhibit significant inter- and intra-familial variation in phenotypic severity making genotype-phenotype correlations difficult. The oim mutation results in deposition of only homotrimeric collagen, $1(I)_3$, leading to an OI type III-like phenotype (short stature and deformation of long bones). To study the impact of genetic background on clinical outcome, we evaluated the *oim* defect on two genetic backgrounds, C57BL/6J (B6) and B6C3Fe a/a. B6 mice are an inbred strain with inherently low bone mineral density and B6C3Fe a/a are an outbred strain used by Jackson Laboratory to propagate *oim* mice. To evaluate geometry and biomechanics, femurs from wildtype (WT) and homozygous (*oim*) animals from each strain were subjected to qCT analyses and torsional loading to failure. Several geometric and biomechanical properties exhibited significant strain and genotype interactions. B6 WT marrow cavity diameter was significantly larger than WT outbred (18% increase), while cortical bone width was significantly decreased in B6 WT as compared to outbred WT (14% decrease). An 18% increase in marrow cavity diameter with a corresponding 9% decrease in cortical bone width was also seen for B6 *oim* as compared to outbred *oim*, though not significant. For each strain, torsional ultimate strength was significantly reduced in *oim* by more than 50% relative to WT. Tensile strength in B6 *oim* was 57% of B6 WT. OH-proline assay showed a 22% decrease in collagen content in outbred *oim* tibias relative to WT, while B6 *oim* tibia collagen was reduced 40% relative to WT. Neutron activation analysis demonstrated significant strain and genotype interactions including alterations in the Ca/P ratios. Taken together, these data indicate a role for genetic background in determination of phenotypic severity for OI.

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Mutation in the *MATN3* gene is common in patients with osteoarthritis. *M. Czarny-Ratajczak[1, 2], A. Kolczewska[3], A. Wozniak[2], A. Latos-Bielenska[2], I. Zimmermann-Gorska[3], D.J. Prockop[1].* 1) Center for Gene Therapy, Tulane University, New Orleans; 2) Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland; 3) Department of Rheumatology, Rehabilitation and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland.

Matrilin 3, one of a group of extracellular matrix proteins, is expressed in the cartilage. Mutations in the *MATN3* gene (2p24-p23), encoding matrilin 3 cause a spectrum of disorders: osteoarthritis, multiple epiphyseal dysplasia and a severe form of spondyloepimetaphyseal dysplasia. Osteoarthritis (OA) is a common debilitating disease involving degeneration of the articular cartilage of joints, particularly hips, knees, lumbar and cervical spine, as well as the distal interphalangeal, the first carpometacarpal and proximal interphalangeal joints of hands.

Fifty consecutive unrelated patients from the Polish population with idiopathic OA were studied. Analysis of the *MATN3* gene revealed that four patients were heterozygous for C to T transition in the codon 303 (T303M), located in the exon 3, that corresponds to the first EGF repeat on the protein level. Two of these patients belong to families with an autosomal dominant form of OA. The T303M substitution was previously detected in patients with hand OA in the Icelandic population and it has been considered as a rare cause (2%) for hand OA. Based on our results, this mutation can account for 8% of all idiopathic OA cases in the Polish population. Analysis of the phenotype of our patients revealed that this mutation causes not only hand OA but also other forms like generalized OA. Currently, we are performing genome-wide screening in four large families with the excluded *MATN3* gene.

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Characterization of craniofacial phenotype in different SMS mouse models. *J. Yan, W. Bi, V.W. Keener, M.J. Justice, J.R. Lupski.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Craniofacial abnormality, by midface hypoplasia, broad nasal bridge, frontal bossing and prognathia, is one of the major clinical manifestations of the Smith-Magenis syndrome (SMS). The majority of the SMS patients have a common 3.7 Mb deletion of 17p11.2, while some with smaller deletions have enabled the refinement of the critical region (SMCR) to an ~1 Mb interval. Recently, mutations have been identified in *RAI1* in patients without FISH detectable deletions. To further investigate the genetic basis of the SMS phenotype, our lab created several mouse models with different deletion sizes including *Df(11)17*, a 2 Mb deletion covering the majority of the mouse region syntenic to the SMS common deletion, *Df(11)17-4* covering the region corresponding to the entire SMCR, and *Df(11)17-1* covering half of the SMCR, as well as *Rai1* knock out mice. Craniofacial abnormalities, including hypertelorism and shorter and curved snouts, have been observed in all of these mouse models, but the penetrance and severity are reduced with the reduced deletion sizes (from 70-80% in *Df(11)17*, to 54% in *Df(11)17-4*, to 46% in *Df(11)17-1*, and 18% in *Rai1*+/- at the N2 generation). Genetic background influences the penetrance as well, with increased penetrance in C57BL/6 background. Skeleton preparations provided quantitative confirmation of visual observations and revealed that the most striking change is the underdeveloped nasal bone. Soft-tissue three-dimensional surface scans of mice is a recently created technique to quantitatively assess the craniofacial changes without sacrificing the mice and provides some measurements which are not possible with skeleton preparation and also enables overall pattern comparison with cluster analysis. With this technique, we confirmed the severity difference in different SMS mouse models and we are now analyzing them in a pure C57 background to see if that will reveal more changes than in the mixed background. To investigate the gene(s) or factors contributing to the variability of craniofacial phenotype in different SMS mouse models will greatly enrich our knowledge of craniofacial development.

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Widely variable phenotype associated with intronic mutation in the NF1 gene. *S. Plotkin[1], M. MacCollin[1], H. Feit[2], J. Gusella[1], T. Callens[3], L. Messiaen[3].* 1) Neurology, Mass General Hospital, Boston, MA; 2) Henry Ford Hospital, Detroit, MI; 3) Dept of Genetics, University of Alabama at Birmingham.

Neurofibromatosis 1 (NF1) is a tumor suppressor gene syndrome associated with a well defined, developmentally expressed phenotype including skin pigmentation changes, nerve sheath tumors, and a range of other manifestations. Over 80% of mutations in the NF1 gene in typical NF1 patients are predicted to grossly truncate or completely eliminate the protein product. A number of recent reports have suggested that a variant or spinal form of NF1 exists and is associated with a different spectrum of mutation including over-representation of missense mutations and C terminal truncating mutations. We report a 24 year old man who presented with massive subcutaneous and deep involvement by neurofibromas at age 16. Most prominently, the entire pelvic contents was replaced by multilobulated tumor growth causing chronic malnutrition and pain. His 25 year old first cousin was identically affected, however both the obligate carrier father and paternal aunt had only painless subcutaneous masses. None of these four individuals had cafe au lait spots, skin fold freckling, typical cutaneous tumors, abnormalities of growth or learning disabilities. Comprehensive RNA- and DNA-based mutational analysis of the NF1 gene revealed an insertion of 47 basepairs between exons 39 and 40 at the cDNA level. Subsequent genomic analysis of this region revealed a substitution at c.7127-770 G>T, creating a strong cryptic splice donor sequence which was present in both cousins and a sibling with several small subcutaneous tumors that had not previously been appreciated. Subtle changes in the NF1 gene appear to result in highly unusual and variably manifesting phenotypes and may hold key insights into the tumor suppressor roles of NF1.

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Mutant glucocerebrosidase provides a link between neurodegenerative disorders. *O. Goker-Alpan[1, 2], D. Urban[1, 2], B.K. Stubblefield[1, 2], E. Sidransky[1, 2].* 1) NSB/NIMH, NIH, Bethesda, MD; 2) MGB/NHGRI, NIH, Bethesda, MD.

Gaucher disease (GD), the deficiency of lysosomal glucocerebrosidase, may provide insights into neurodegenerative disorders characterized by protein misfolding and aggregation. Immunofluorescence studies showed that both mutant glucocerebrosidase and alpha-synuclein were present in Lewy bodies and neuronal inclusions in brain samples from patients with GD and parkinsonism. Moreover, glucocerebrosidase mutations have been identified in subjects with a spectrum of synucleinopathies. In these disorders, mutant glucocerebrosidase may serve as a risk factor, leading to aggregate formation either by impairing proteosome function or by interfering with synuclein clearance. To test the first hypothesis, ubiquitination of glucocerebrosidase-reactive inclusions were studied in brain tissues from subjects with GD and parkinsonism. Mutant glucocerebrosidase was identified in both ubiquitinated and non-ubiquitinated aggregates. The non-ubiquitinated aggregates stained with antibodies against the lysosomal markers, suggesting the role of both ubiquitin-dependent proteolysis and lysosomal degradation in inclusion formation. Additional evidence that mutant glucocerebrosidase might cause proteosomal dysfunction was provided by examining molecular chaperones in fibroblast cell lines. Levels of HSP90, the primary chaperone preventing aggregation of misfolded proteins and maintaining proteosome integrity, were decreased in cell lines carrying missense mutations. In the HSP90-deficient fibroblasts, levels of another chaperone, Hsc-70, and its receptor, LAMP2, were also reduced. Hsc-70 and LAMP2 levels are rate-limiting for chaperone mediated autophagy (CMA) in the lysosomes. As alpha-synuclein is degraded by the CMA pathway, this finding suggests that mutant glucocerebrosidase may interfere with synuclein degradation as well. Thus, mutations in glucocerebrosidase may impact different pathways implicated in the synucleinopathies. Further studies of the relationship between alpha-synuclein and mutant glucocerebrosidase may advance our understanding of the pathogenesis of common neurodegenerative disorders.

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Compound heterozygosity of *LHX3* in a patient with septo-optic dysplasia. *A. Campos-Barros, L. Soriano-Guillen, K.E. Heath, J. Argente.* Endocrinology, Hospital Infantil Universitario Niño Jesús, Madrid, SPAIN.

Septo-optic dysplasia (SOD) is a clinically heterogeneous disorder defined by any combination of optic nerve hypoplasia, pituitary gland hypoplasia and midline abnormalities of the brain, including absence of the corpus callosum and septum pellucidum. SOD is often associated with combined pituitary hormone deficiency (CPHD). Mutations in *HESX1* have been found in homozygosity in severe SOD and in heterozygosity in mild SOD. However, incomplete penetrance has been observed, and in many SOD cases the molecular bases remains unknown.

We present the case of a short stature (P3) female proband (4 years and 11 months old at diagnosis) born by caesarean delivery after a 38 weeks pregnancy. As a newborn she was transferred to an ICU because of severe transient neonatal hypoglycaemia and shock. Cranial MRI studies revealed the absence of the septum pellucidum together with optic nerves and chiasma atrophy. Hormonal tests revealed hypothyroidism and TSH deficiency at the age of 6 months, GH deficiency with low growth velocity (P3) at age 5, and undetectable ACTH at 8 years, respectively, compatible with a diagnosis of CPHD associated to SOD. Molecular studies excluded the presence of mutations in the coding sequences and intron/exon boundaries of *HESX1*, *PROP1*, *POU1F1* and *LHX4*, by dHPLC and DNA sequencing. Screening of *LHX3* detected two previously undescribed mutations in the proband: a missense mutation in exon 6 at a phylogenetically conserved residue, R309P, and a c1494CA conversion in the 3'-UTR of the *LHX3* transcript, inherited from the unaffected father and mother, respectively. None of the new variants was observed in 100 chromosomes from healthy controls, nor in a group of 35 CPHD patients.

To our knowledge this is the first case of SOD which is associated with *LHX3* mutations. Two recessive mutations in *LHX3*, which encodes a LIM class homeodomain protein member, have been previously linked to rare cases of CPHD presenting with GH, TSH, PRL, LH and TSH deficiency and a rigid cervical spine. Functional analysis will characterize the compound pathogenicity of these two novel mutations. (acamposbarros@yahoo.es).

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Clearance of tau oligomers by the proteasome is critically dependent on the Tau phosphorylation state in Drosophila. *O. Blard[1], A. Boutajangout[2], J-P. Brion[2], D. Campion[1], T. Frebourg[1], M. Lecourtois[1].* 1) Inserm U614, Faculty of Medicine, 22 boulevard Gambetta, 76183 Rouen, France; 2) Laboratory of Histology, Neuropathology and Neuroanatomy, Universite Libre de Bruxelles, School of Medicine, 808 route de Lennik, B-1070 Brussels, Belgium.

In tauopathies, including Alzheimers disease (AD) and fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), microtubule associated protein Tau is abnormally hyperphosphorylated and is accumulated as intraneuronal tangles of paired helical filaments (PHF). In AD brains, it has been shown that the level of Tau is higher than in age-matched control brains, and this increase concerns abnormally hyperphosphorylated Tau. Using Drosophila as a model of tauopathies, we studied the relationship between Tau phosphorylation and degradation. We showed that genetic inactivation of the proteasome specifically induces an accumulation of oligomeric forms of highly phosphorylated Tau. The clearance of these Tau oligomers by the proteasomal system is critically dependent on the Tau phosphorylation state. Finally, we found that impairment of proteasome activity or deregulation of Tau phosphorylation leads, concomitantly to the accumulation of oligomeric forms of highly phosphorylated Tau, to the formation of filaments.

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A new locus for a progressive autosomal recessive spastic ataxia in the French-canadian population: clinical description and genome-wide scan analysis. *I. Thiffault[1,2], M.F Rioux[1], M. Tetreault[1,2], J. Jarry[1,2], J. Poirier[2], L. Loiselle[2], J. Mathieu[3], J.P. Bouchard[4], G.A. Rouleau[2], B. Brais[1,2].* 1) Neurogenetics,CHUM-Notre Dame Hospital, Montreal, QC, Canada; 2) Centre for the Study of Brain Diseases, Notre-Dame Hospital, CRCHUM, Montreal, QC, Canada; 3) Department of Neurology, Sagamie, Saguenay, QC, Canada; 4) Department of Neurology, CHUL, Quebec, QC, Canada.

Recessive spastic ataxias are a heterogeneous group of neurodegenerative diseases. Relying on a network of Ataxia and Neuromuscular clinics we were able to identify familial cases of an original form of autosomal recessive spastic ataxia. We identified a group of 25 cases belonging to 19 families that share clinical similarities. More than 50% of families have a genealogical relationship with the Portneuf County south of Quebec City. The major clinical features are the presence of spasticity, hyperreflexia, ataxia and dysarthria. The age of diagnosis is variable (mean 15.2 years, range 2-59). The more severe cases have severe spasticity from birth, scoliosis, dystonia and cognitive impairment and are often considered cases of cerebral palsy. There is a great intrafamilial and interfamilial variability in the severity of the phenotype. All MRI show cerebellar atrophy. In the more severe cases cortical atrophy, leukodystrophy and corpus callosum thinning are also observed. A genome scan uncovered linkage of three informative families to a candidate region of 15cM (multipoint LOD score of 2.08). Linkage analysis confirmed that at least 15 families are linked to the same region (multipoint LOD score of 5.76). Haplotype analysis suggests that the two most common mutations would account for approximately 85% carrier chromosomes. The identification of the mutated gene in will help the genetic counseling for this disease in French-Canadian population. The identification of the mutated gene will likely also shed light on key pathways underlining cerebellar and other central nervous system development and degeneration because of the significant clinical overlap between this form of ataxia and paraparesis, cerebral palsy and leukodystrophies.

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Identification of the SCA28 locus for a novel form of autosomal dominant spinocerebellar ataxia. *D. Di Bella[1], C. Cagnoli[2], C. Gellera[1], C. Mariotti[1], M. Seri[3], P. Pappi[2], M. Plumari[1], R. Fancellu[1], A. Brussino[2], N. Migone[2], S. DiDonato[1], F. Taroni[1], A. Brusco[2].* 1) Div Biochemistry & Genetics, Istituto Neurologico C Besta, Milan, Italy; 2) Dept Genet Biol Biochem, University of Torino, Italy; 3) Dept Internal Med, University of Bologna, Italy.

The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of autosomal dominant neurodegenerative disorders characterized by progressive cerebellar ataxia associated with other neurological features. Molecular studies in SCAs indicate a large genetic heterogeneity and, at present, 26 distinct loci have been mapped, while the disease genes have been identified for only 12 forms. In 6 SCA subtypes and DRPLA the molecular mutation is characterized by CAG/CTG repeat expansion; however, known SCA genes appear to be responsible for only 40-60% of familial cases. We have identified a 4-generation family (29 subjects, including 11 affected members) with a novel form of autosomal dominant cerebellar ataxia, characterized by juvenile onset, slowly progressive ataxia, and oculomotor abnormalities including nystagmus and ophthalmoparesis. Genetic analyses excluded known SCA genes and loci (SCA1-8, 10-23, 25-27, and DRPLA). RED analysis did not reveal CAG/CTG expansions larger than 40 repeats (Brusco et al, Arch Neurol 2004). A genome wide study suggested linkage to a new locus on chromosome 18. Pairwise and multipoint linkage analyses obtained a maximal LOD score of Z=4.77. Haplotype reconstruction showed that a single haplotype segregated with the disease in the family and defined the minimal region in linkage as a 7.9 Mb region which encompasses approx. 70 genes. This new locus has been assigned the SCA28 symbol by the HGNC (www.gene.ucl.ac.uk/hugo). A number of interesting candidate genes have been selected and sequence analysis of the coding exons is currently underway. This disorder represents a novel form of autosomal dominant spinocerebellar ataxia mapped to chromosome 18 and identification of the responsible gene will provide further insight into mechanisms of neurodegeneration. (Supported by grants: Telethon GGP04254 to AB, EuroSCA to SDD, RF2002/160 to FT).

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BI-1, an mBattenin/Battenin-interactive partner protein, is exclusively mislocalized at nuclei in Batten disease. *N. Zhong, W. Ju, J. Shen, W.T. Brown.* Dept Human Genetics, New York State Inst Basic Res, Staten Island, NY.

We have earlier identified Bax inhibitor-1 (BI-1) protein as an mBattenin/Battenin-interactive partner protein. We have found that the mutant Battenin (mBattenin) has an increased binding affinity with BI-1 compared to Battenin. To investigate whether BI-1 is affected by the differential binding activity in Batten disease, we undertook studies with immunostaining of BI-1 in both wild type and mutant fibroblasts. Our data showed that in wild type fibroiblasts, BI-1 predominantly distributes in the endoplasmic reticulum (ER). However, it is exclusively localized at nuclei in the mutant fibroblasts derived from Batten disease. To investigate if the mislocalization of BI-1 seen in the mutant fibroblasts is also seen in neuronal cells, immunostaining of endogenous BI-1 was conducted in human neuron precursor NT2 cells that stably express mBattenin, compared to control cells. Our results confirmed that normal BI-1 is localized at ER in control NT2 cells. However, in mBattenin-expressing NT2 cells, there was no detectable BI-1 at the ER, rather the anti-BI-1Ab strongly stained the nuclei. To further investigate if the mislocalization of BI-1 at the nuclei results from a gain-of-function due to the expression of truncated exogenous mBattenin or to the loss-of-function of endogenous normal Battenin, we undertook knock down experiments with siRNA approach. Our studies showed that the siRNA specific targeting of endogenous Battenin in NT2 cells resulted in the same outcome, that BI-1 is exclusively mislocalized at nuclei. To further quantitatively analyze the distribution of BI-1 in NCL3 cells, total protein from nuclei, ER, and cytosol was extracted from NCL3 and control fibroblasts. The BI-1 signal in the fraction of nuclei isolated from NCL3 patient fibroblasts is much stronger than in normal controls, while it is weaker in the ER of mutant cells and not significantly different in the cytosol comparing normal and mutant cells. Our results indicated that the anti-apoptotic agent BI-1 is affected in the Batten disease.

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The genetically dystonic rat harbors a novel *Atcay* insertional mutation. *J. Xiao, M.S LeDoux.* Departments of Neurology and Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN.

The genetically dystonic rat (SD-dt:JFL), an autosomal recessive animal model of primary generalized dystonia discovered in the Sprague-Dawley strain, develops a dystonic motor syndrome by P12. Dystonic rats exhibit axial and appendicular dystonia that progresses in severity with increasing postnatal age. Dystonia is reduced when the animals are at rest and disappears during sleep. Without surgical intervention, dt rats die prior to P40 despite gavage feedings and other supportive measures. The mutation is fully penetrant and there is negligible variability to its expressivity. Mutations in the coding region of the gene (*DYT1*) associated with Oppenheims dystonia have been excluded in the dt rat. A constellation of morphological, pharmacological, neurochemical, and neurophysiological studies have identified olivocerebellar pathways as a site of functional abnormality in the dt rat. To find the mutant gene, a post-cerebellectomy male dt rat was crossed with a female of an inbred strain (SHR) to create F1 heterozygotes. Next, F1 rats were crossbred to yield 875 F2 offspring. After the first round of genotyping, three microsatellite markers on Chr 7 (D7Rat36, D7Rat35, and D7Rat32) showed strong linkage to the dystonia phenotype. Relative distances from the telomeric end of Chr 7 (0.04 cM, 6.8 cM, and 11.6 cM, respectively) and recombination rates (0%, 2.9%, and 8.7%, respectively) indicated that microsatellite marker D7Rat36 was nearest the causal mutation. Additional microsatellite markers near D7Rat36 were selected from a genetic linkage map based on the SHRSP x BN cross. Refined mapping was performed with these additional markers in 875 F2 rats. The dt locus was mapped to a 4.2 Mb region on Chr 7q11 (between D7Rat188 and D7Rat113) and candidate genes were screened with semi-quantitative RT-PCR. Then, Southern blotting and genomic DNA sequencing identified the 3-long terminal repeat portion of an intracisternal A particle element inserted into Intron 1 of *Atcay*, the gene which encodes caytaxin. Northern and Western blotting and quantitative real-time RT-PCR defined the Atcay allele in dt rats ($Atcay^{dt}$) as hypomorphic.

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The elucidation of cell compartmentalization and spatial expression pattern of the paroxysmal non-kinesigenic dyskinesia gene. *H.-Y. Lee[1], Y. Xu[1], J. Nakayama[1], R. Tu[1], Y.-H. Fu[1], L.J. Ptacek[1,2].* 1) Department of Neurology, University of California, San Francisco, CA; 2) Howard Hughes Medical Institute, University of California, San Francisco, CA.

Paroxysmal non-kinesigenic dyskinesia (PNKD) is an autosomal-dominant disorder that belongs to a group of hyperkinetic movement disorders called the paroxysmal dyskinesias. We have identified two missense mutations in a novel gene called Myofibrillogenesis Regulator 1 (MR-1) located on chromosome 2q, which caused PNKD in 50 affected individuals from eight kindreds. There are three isoforms of MR-1, named long (L), medium (M), and short (S). These mutations caused alanine-to-valine changes in the N-terminus of MR-1L and MR-1S isoforms. Two antibodies for MR-1 were made by using two synthesized oligopeptides which corresponded to the predicted amino-terminus of MR-1L and S, and the predicted carboxy-terminus of MR-1L and M. These antibodies can also recognize the ortholog of MR-1 in mouse (mMR-1). Western blot analysis shows that mMR-1L is expressed exclusively in brain, and the mMR-1M is widely expressed in different tissues. Immunohistochemistry using anti-MR-1 antibodies indicates that mMR-1 is widely expressed in neuronal cells throughout the mouse central nervous system (CNS). The expression of mMR-1 in the motor cortex, cerebellum, spinal cord, and basal ganglia, implies that MR-1 plays an important role in maintaining the excitability of these essential parts of the motor system. Based on the sequence homology, we predict that MR-1 belongs to the glyoxalase family. Transfection of EGFP fusion protein construct of MR-1L in HEK293 cells shows that the MR-1L is localized to membrane in this heterologous system and this membrane localization feature is unique from any known glyoxalase. By transfecting EGFP constructs of MR-1L followed by an immunohistochemistry study of permeable or non-permeable HEK293 cells; we find that both the N-terminus and C-terminus of MR-1L are located inside the cell. Further experiments are underway to examine possible differences in mMR-1 expression in the CNS between wild-type mice and a transgenic mouse model of PNKD.

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A Phosphorylation Cascade Regulating Circadian Period in Mammals. *Y. Xu[1], C.R. Jones[2], K.L. Toh[1], Q.S. Padiath[1], L.J Ptacek[1,3], Y.--H Fu[1].* 1) Neurology, University of California, san francisco, CA; 2) Department of Neurology, University of Utah, Salt Lake City, Utah 84132-2450; 3) 3Howard Hughes Medical Institute, University of California, San FranciscoŸŸŸ.

The behavior and physiology of most organisms is subject to circadian (24 hr) rhythmicity. Negative-feedback loops in clock genes are though to control circadian period. Familial advanced sleep phase syndrome (FASPS) in humans manifests with extreme early sleep onset times and early morning awakening. We previously reported a missense mutation (S662G) in the Period 2 (PER2) protein causing FASPS in one family and resulting in hypophosphorylation in vitro. Here we engineered mice containing a human Per2 S662 transgene. Mice transgenic for S662G recapitulate the short period FASPS phenotype seen in humans. Mice with a transgene encoding an acidic residue at position 662 showed period elongation. These and additional biochemical experiments demonstrate the charge on residue S662 regulates a phosphorylation cascade of downstream serines that critical for determination of circadian period. This represents direct in vivo demonstration that phosphorylation of PER2 regulates circadian period in mammals and defines a critical role for PER2 in circadian period length determination. Further analysis of these human animal models will provide us with a unique opportunity to understand the regulation of the circadian clock and its effect on various behavioral phenotypes.

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Spastic paraplegia type 2 associated with axonal neuropathy and apparent *PLP1* position effect. *K. Inoue[1,2], J.A. Lee[2], R.E. Madrid[3], K. Sperle[4], C. Ritterson[5], G.M. Hobson[4,6], J. Garbern[7], J.R. Lupski[2,8,9].* 1) Dept Mental Retardation & Birth Defect Research, National Institute Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas; 3) NYS Institute for Basic Research in Developmental Disabilities, George A. Jervis Clinic, Staten Island, New York; 4) Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Nemours Children's Clinic, Wilmington, Delaware; 5) Department of Biology, Boston University, Boston, Massachusetts; 6) Department of Pediatrics, Jefferson Medical College, Philadelphia, Pennsylvania; 7) Department of Neurology and Center of Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan; 8) Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 9) Texas Childrens Hospital, Houston, Texas.

Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type 2 (SPG2) are clinically distinct allelic disorders, yet represent a wide spectrum of central nervous system dysmyelination associated with mutations in the Xq22.2 proteolipid protein gene (*PLP1*). Approximately 80-90% of PMD/SPG2 patients have *PLP1* mutations, including duplication, deletion, and point mutations in exons or splice junctions. Interestingly, although *PLP1* deletion mutations are very rare, they cause a more extended nervous system clinical phenotype that includes peripheral neuropathy. We report an association between SPG2 with axonal peripheral neuropathy and apparent *PLP1* gene silencing in a family. Electrodiagnostic studies and a sural nerve biopsy revealed features of a chronic axonal neuropathy. Pulsed-field gel electrophoresis, custom array comparative genomic hybridization, and semi-quantitative multiplex PCR analyses identified a small duplication downstream of *PLP1*, which we propose to result in *PLP1* gene silencing by virtue of a position effect. Our observations further suggest that genomic rearrangements that do not include *PLP1* coding sequences should be considered as yet another mutational mechanism underlying *PLP1*-related dysmyelinating disorders.

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SIX3 and ZIC2 mutations in a series of holoprosencephaly patients. *J. Herbergs[1], S. Spierts[1], D. Tserpelis[1], A. Van Haeringen[2], M. Kerstjens-Frederikse[3], G. Mancini[4], M. Kwee[5], J. Hoogeboom[4], I. Stolte-Dijkstra[3], H. Smeets[1].* 1) Dept Clinical Genetics, Academic Hosp Maastricht, Maastricht, Netherlands; 2) Leiden University Medical Centre, Leiden, The Netherlands; 3) University Medical Center Groningen, The Netherlands,; 4) Erasmus Medical Center, Rotterdam, The Netherlands; 5) VU University Medical Center, Amsterdam, The Netherlands.

Holoprosencephaly (HPE) is a common severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. The prevalence is 1:250 during early embryogenesis, but the live born prevalence is only 1:16000. The etiology of HPE is extremely heterogeneous and can include both a teratogenic and/or genetic basis. We studied four genes known to be involved in HPE, namely SHH, ZIC2, SIX3 and TGIF by sequence analysis. A series of in total 31 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 7 pathogenic mutations (23%), 5 out of 28 sporadic cases (18%) and 2 out of 3 familial cases (67%). Three mutations were detected in the SIX3 gene and four mutations in the ZIC2 gene. A ZIC2 mutation in a sporadic case appeared not to be a de novo mutation, but was also found in a healthy mother with no clinical features of the HPE spectrum. This was also seen in a carrier of the mutation in a familial case. In this study the genetic heterogeneity of the disease and the extremely variable phenotypes in HPE families have been confirmed.

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Proteasome function in tauopathies. *A. Lecrux[1], D. Campion[1], J. Bou[1], J-B. Latouche[1], T. Frébourg[1,2], C. Dumanchin[1].* 1) Inserm U614, IFRMP, Faculty of Medicine, Rouen; 2) Department of Genetics, Rouen University Hospital, Rouen, France.

Tauopathies are neurodegenerative diseases caracterized by intraneuronal accumulation of hyperphosphorylated Tau protein (MAPT), into paired helical filaments (PHF) or straight filaments, leading to neuronal death by an unknown mechanism. Frontotemporal Dementia and Parkinsonism linked to chromosome 17 (FTDP-17) are primary tauopathies resulting from Tau gene mutations. These mutations have different consequences on Tau function: an increase of its aggregability, a decrease of its affinity to microtubules and of its ability to induce their polymerization. Secondary tauopathies, like Alzheimers Disease, result from an alteration of Tau phosphorylation. In several neurodegenerative diseases, like familial Parkinsons Disease and polyglutamine diseases like Huntington disease, it has been suggested that neuronal death can result in part from a dysfunction of the Ubiquitin Proteasome System (UPS), leading to a decrease of proapoptotic protein degradation. When the proteasome is overflowed, proteins are targeted to the centrosome where they form a structure: the agresome. However, the implication of the UPS in neurodegenerative diseases remains controversial. We hypothesized that the neuronal death observed in tauopathies could also result from an alteration of UPS function and therefore investigated the effect of Tau accumulation on UPS function. We studied two mutations: P301L and V337M, which induce an increase of Tau aggregation in FTDP-17, and determined the effect of the overexpression on UPS function using retroviral infection. To study UPS function, we established a clonal line of neuronal cells (SH-5YSY) stably expressing EGFP-degron, a reporter developed by Ron Kopito, consisting of a short degron fused to the COOH terminus of EGFP. We have expressed Tau protein during 12 days and the proteasome was not impaired in basal conditions. To determine the effect of Tau hyperphosphorylation in this system, wild type and mutant tau proteins and the kinases GSK3 and Cdk5 are being coexpressed, using the same reporter.

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CKN1 gene analysis in Cockayne syndrome: novel mutations in eight typical patients. *D. Bertola[1], H. Cao[2], L.M.J. Albano[1], D.P. Oliveira[3], F. Kok[3], M.J. Marques-Dias[3], C.A. Kim[1], R.A. Hegele[2].* 1) Genetics Unit, Instituto da Crianca, Sao Paulo, Brazil; 2) Robarts Research Institute, Ontario, Canada; 3) Neurology, University of Sao Paulo, Sao Paulo, Brazil.

Cockayne syndrome is a rare autosomal recessive neurodegenerative disorder. It is considered to be a heterogeneous condition based on complementation in cell-fusion studies, with three major forms, namely CS-A, CS-B and CS-C. CKN1 is the gene responsible for CS-A, whose mutations disrupt the transcription-coupled repair system of the actively transcribed DNA. Mutation analysis of the CKN1 gene in eight typical CS-A Brazilian patients from six families showed a gene alteration in all of them. Six novel different unreported mutations were found and only one pair of siblings was a compound heterozygote. While the findings extend the range of mutations in CS-A, there is no obvious genotype-phenotype correlation across the mutational spectrum.

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Apparent transgenerational stability of CTG trinucleotide repeats in 2 families of women with myotonic dystrophy. *D. Genevieve[1], M. Jambou[1], G. Gourdon[2], J. Martinovic[1], S. Loizeau[3], M. Vekemans[1], A. Benachi[3], Y. Dumez[3], M. Masri[4], A. Munnich[1], J.P. Bonnefont[1].* 1) Dept de Genetique, Hosp Necker-Enfants Malades, Paris, France; 2) Unite INSERM U383, Hopital Necker-Enfants Malades, Paris, France; 3) Service de Gynecologie Obstetrique, Hopital Necker-Enfants Malades, Paris, France; 4) Service de Gynecologie Obstetrique, Hopital François Mitterrand, Pau, France.

Myotonic dystrophy (MD) is a frequent neuromuscular disease due to instability of CTG trinucleotide repeats in the 3 untranslated region of the dystrophia myotonica protein kinase (DMPK) on chromosome 19q13.3. Normal individuals have 5 to 37 CTG repeats which remain stable across generations while affected individuals have over 50 CTG repeats. In the majority of DM patients, the number of repeats is highly unstable and increases following transmissions of the mutant allele, providing molecular bases to the anticipation phenomenon. Here, we report on apparent intergenerational CTG repeat stability in two families of MD females. The number of the CTG repeats in blood cells was estimated at 143, 133, 121 and 109 for the great-grand mother, grandmother, mother and daughter, respectively in the first family and 350, 300 and 200 for the grand mother, mother and fetus respectively in the second family.Interestingly, the absence of intergenerational CTG expansion correlated with the absence of clinical anticiption We have had the opportunity to analyse the number of CTG repeats in several fetal tissues after termination of pregnancy in an affected fetus in each family at 15 weeks of gestation. The size of the CTG repeats was strictly identical in muscle, brain, liver, heart and chorion villi in both fetuses. These data suggest the existence of a dominantly inherited factor preventing the instability of the expanded CTG stretch during female gametogenesis in these families. Based on data from a MD murine model suggesting that DNA mismatch repair genes could account for CTG repeats contraction (Savouret et al,. Mol Cell Biol. 2004 24:629-37), molecular and protein studies of MSH2 and MSH3 genes are in progress in these families.

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Decreased expression of Hsp27 and Hsp70 in transformed lymphoblastoid cells from patients with Spinocerebellar Ataxia Type 7. *S.Y. Li[1], M. Hsieh[2], S.J. Lin[3], C. Li[4], H.F. Tsai[5].* 1) Genetics Lab, Dept Life Sci, Chung Shan Medical Univ, Taichung, Taiwan; 2) Dept Life Sci, Tunghai Univ, Taichung, Taiwan; 3) Dept of Medicine, College of Medicine, National Cheng Kung Univ, Tainan, Taiwan; 4) Dept Life Sci, Chung Shan Medical Univ, Taichung, Taiwan; 5) School of Medical Technology, Chung Shan Medical Univ, Taichung, Taiwan.

Spinocerebellar ataxia type 7 (SCA7) is caused by an expansion of unstable CAG repeats within the coding region of the novel gene, ataxin-7, on chromosome 3. This disease is also associated with an accumulation of abnormal proteins, including expanded polyglutamine-containing proteins, molecular chaperones, and the ubiquitin-proteasome system. In this study, two SCA7 lymphoblastoid cell lines (LCLs) with 100 and 41 polyglutamine repeats were utilized to examine the effects of polyglutamine expansion on heat shock proteins. Interestingly, under basal conditions, Western blot and immunocytochemical analysis showed a significant decrease of Hsp27 and Hsp70 protein expression in cells containing expanded ataxin-7, as compared with that of the normal LCL. On the other hand, the protein levels of Hsp60 and Hsp90 were not significantly altered in the mutant LCLs. Results from semi-quantitative RT-PCR indicated that the differences in Hsp70 protein levels were due to transcriptional defects while the reduction of Hsp27 in the mutant cells was not caused by transcriptional defects. Our results further demonstrated that despite of defective protein expression of Hsp27 and Hsp70, a normal heat shock response was observed in lymphoblastoid cells expressing mutant ataxin-7. Taken together, our results indicated that expanded ataxin-7 that leads to neurodegeneration significantly impaired the expression of Hsp27 and Hsp70 protein, which may be, at least in part, responsible for the toxicity of mutant ataxin-7 proteins and ultimately resulted in an increase of stress-induced cell death.

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***E. coli ssb* stabilizes GAA triplet-repeat sequences.** *L. Pollard, S. Bidichandani.* Dept Biochem & Molec Biol, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK.

Friedreich ataxia is caused by the expansion of a GAA triplet-repeat (GAA-TR) sequence in intron 1 of the *FRDA* gene. This expanded GAA-TR is unstable in somatic cells, with a predilection for contractions, but the mechansim for this instability is poorly understood. Using *E. coli* as a model system, we have previously found that the GAA-TR is more unstable, with a marked contraction bias, when "GAA" is the template for lagging strand synthesis and that replication is impeded in this orientation. We propose that the mechanism for the observed contraction bias is related to the known ability of single-stranded GAA sequence to form secondary structures, thus being bypassed during lagging strand replication. We found that interruptions in the GAA-TR tract, which prevent the sequence from forming secondary structures, stabilize the repeat. Since replication protein A (RPA), which stabilizes the single-stranded lagging strand template during replication, binds to purine-rich sequences with 50-fold lower affinity than pyrimidine-rich sequences, we propose that contractions of the GAA-TR in somatic cells occur when "GAA" is the lagging strand template, due in part to an increased capacity for stable secondary structure formation. To test this hypothesis, we replicated GAA-TR sequences in an *E. coli* strain harboring a temperature-sensitive mutation in single-stranded binding (*ssb*) protein (RPA homolog). By comparing the level of GAA-TR instability in this strain to that in the isogenic wild-type strain at the non-permissive temperature, we observed a dramatic length-dependent increase in instability when *ssb* is mutated. Instability was also orientation-dependent; when *ssb* is inactive, the GAA-TR is significantly more unstable when "GAA" is the lagging strand template. However, this difference in instability did not appear to coincide with a disparity in the replication rate between the two orientations. These results indicate that although mutating *ssb* may not affect the replication rate of GAA-TR sequences, *ssb* does play an important role in reducing instability during replication, especially when "GAA" is the template for lagging strand synthesis.

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Alteration of the Microtubule-Associated Proteins mRNA levels by CTG repeats effect. *P. Velazquez-Bernardino[1,2], M. Bermudez[2], F. Garcia-Sierra[1], B. Cisneros[2].* 1) Dept Cell Biology; 2) Dept Genetics & Mol Biol, CINVESTAV-IPN, Mexico, D.F., Mexico.

Myotonic dystrophy (DM) is a dominant neuromuscular disorder caused by expansion of the CTG trinucleotide repeat tract localized in the 3'-untranslated region (3'-UTR) of the DMPK gene. To address the effect of the DM gene mutation on neuronal function, we isolated cell clones derived from PC12 neuronal cell line, which express the 3'-UTR DMPK region with 90 CTG repeats (CTG90 clone). Previously, we found that 90 CTG repeats expression provokes an inhibition of the NGF-induced PC12 cell neuronal differentiation. In order to identify neuronal genes that could be altered by the DM mutation, we have analyzed the gene expression of the CTG90 clone using the cDNA microarray technology. We found altered expression of several microtubule-associated proteins (MAP1A, MAP1B, MAP2, MAP6, Tau and Doublecortin). We confirmed by real time PCR that MAP2 mRNA expression is decreased in the CTG90 clone, as compared with the wild type cell line. At the present stage of this investigation, it is difficult to identify what level of the MAP2 gene expression is altered by the CTG repeat expansion. Recently, it was found that mutant DM transcripts sequestered Sp1 and Sp3 transcription factors. At this respect, it is important to mention that the MAP2 promoter sequence revealed two Sp1 binding sites. Therefore, it is tempting to speculate that the CTG repeat expansion affects MAP2 expression at promoter level by interfering with the action of Sp1. As a first step to test this hypothesis, we decided to evaluate the effect of Sp1 over-expression on MAP2 mRNA levels in the wild type PC12 cells. We found an increased MAP2 mRNA expression, which suggests that Sp1 indeed regulates positively the MAP2 promoter activity. Given the importance of MAP2 as structural components of neurites, it is likely that mutant CTG repeats inhibits PC12 neuronal differentiation by affecting its expression. This work is supported by the Muscular Dystrophy Association, Inc. Grant No. MDA-3693.

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A new locus for autosomal dominant hereditary spastic paraplegia (SPG29) maps to chromosome 2p12. *A. Ashley-Koch[1], M.E. Kail[1], M.A. Nance[2], P.C. Gaskell[1], I.K. Svenson[3], D.A. Marchuk[3], M.A. Pericak-Vance[1], S. Züchner[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Park Nicollet Clinic, Minneapolis, MN 55415, USA; 3) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

The hereditary spastic paraplegias (SPG) are a group of neurodegenerative diseases clinically characterized by progressive lower limb spasticity and paresis of varying severity. Clinical heterogeneity in the form of additional neurological symptoms and varying degrees of severity as well as varying age of onset is supported by an impressive genetic heterogeneity. To date, 10 chromosomal loci have been reported causing autosomal dominant forms of SPG and five of the underlying genes have been identified. However, additional genetic heterogeneity has been suggested. We conducted a whole genome linkage screen on two independent families and identified a new SPG locus on chromosome 2p12. The combined two-point LOD score was 4.7 at marker D2S2951. Fine mapping with additional microsatellite markers narrowed the region to about 9 Mb between D2S139 and D2S2181. Within that region we identified several candidate genes that share domains or pathways with known SPG genes. Thus far, we have excluded causative mutations in the genes vesicle-associated membrane protein 5 (VAMP5), VAMP8, vacuolar protein sorting 24 (VPS24), and the trans-Golgi network protein (TGOLN2).

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A single nucleotide substitution in the 5'f-UTR in the gene encoding spectrin repeat and Rho guanine nucleotide exchange factor domains is strongly associated with autosomal dominant cerebellar ataxia linked to chromosome 16q22.1. *K. Ishikawa[1], S. Toru[1], T. Tsunemi[1], T. Amino[1], K. Kobayashi[2], I. Kondo[3], T. Toda[2], H. Mizusawa[1].* 1) Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept Clinical Genetics, Osaka Univ., Suita, Japan; 3) Dept Medical Genetics, Ehime Univ, Ehime, Japan.

Autosomal dominant cerebellar ataxia (ADCA) is a group of heterogeneous neurodegenerative diseases showing progressive cerebellar ataxia as a cardinal clinical feature. We mapped families with 'gpure'h cerebellar ataxia to a 3 cM region in the chromosome 16q22.1 (Nagaoka et al.,2000). This region was the locus for another ADCA, spinocerebellar ataxia type 4 (SCA4)(Flanigan et al., 1996), which shows prominent peripheral neuropathy. Unlike SCA4, our families showed progressive ataxia confined to cerebellar nature, although some patients show hearing impairment. To identify the gene for our families with ADCA linked to chromosome 16q22.1, we constructed a physical map of the locus and analyzed haplotypes for newly discovered polymorphic DNA markers in our 50 families recruited all over Japan. This approach allowed us to identify the critical interval into <600 kb region with a common haplotype determined with 4 polymorphic markers in 16q22.1. Within this region we found a C-to-T single-nucleotide change in the 5'f-UTR in the gene, puratrophin-1. This genetic change, resided 16 nucleotides upstream of the tranlation initiation codon, was seen in all affected individuals from all 50 families, but was not seen in 1000 control Japanese chromosome. The in vitro luciferase assay showed that this C-to-T change results in significantly reduced transcription efficacy. Supporting this observation, a tendency for reduction of puratrophin-1 mRNA expression was seen in patients'f frozen cerebellar samples. Interestingly, immunohistochemistry for puratrophin-1 demonstrated formation of microscopic aggregation in Purkinje cells of patients'f cerebelli. Golgi apparatus associated protein and spectrins were also aggregated. These observations may suggest that the C-to-T change in the 5'f-UTR is strongly associated with ADCA linked to 16q22.1.

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Development of a DNA microarray-based high-throughput gene analysis system for Parkinson disease. *N. Seki, Y. Takahashi, J. Goto, S. Tsuji.* Department of Neurology, The University of Tokyo, Tokyo.

[Introduction] Parkinson disease (PD) is a common neurodegenerative disorder characterized by extrapyramidal symptoms caused by neuronal loss in substantia nigra. The causative genes for hereditary PD thus far identified are *SNCA*, *PARK2*, *UCHL1*, *DJ1*, *NR4A2*, *LRRK2*, and *PINK1*. Occasionally, the mutations of these genes are also identified in apparently sporadic cases. In addition, accumulating evidences suggest that sporadic PD is a polygenic disease, where multiple genes play essential roles in the pathogenesis.With this background, comprehensive screening of these causative and candidate genes is mandatory not only for hereditary PD but also to explore genetic components involved in the pathogenesis of sporadic PD. The purpose of this study is to develop a high-throughput system that allows mutational analysis of causative and related genes for Parkinson disease employing a DNA microarray-based resequencing platform. [Method] DNA microarrays TKYPD01 and TKYPD02 were designed based on a microarray-based resequencing system(Affymetrix). These microarrays contain all the causative genes for PD as described above and for related disorders (*GCH1* and *DYT1*), as well as PD-related genes (*TH*, *SNCAIP*, *GPR37*, *APOE*, *CHT1*, *GBA* and *MTX1*). The promoter sequence and intronic sequences of *SNCA* were also included. To validate the accuracy of nucleotide sequence analysis, genomic DNA samples from a normal control and 6 patients with PD were subjected to specific PCR amplification and processed. In order to handle the numerous PCR products, a robotic system was employed. [Result]All the causative and PD-related genes for PD could be analyzed simultaneously, and the accuracy of base calls was 99.94% (18897 bases out of 18908). 7 known SNPs and 7 novel SNPs(1 nonsynonymous, 2 synonymous, and 4 non-coding) were identified in these samples. [Conclusion]The microarray-based resequencing system is a highly efficacious and reliable system for mutational analysis and identification of SNPs of the causative genes as well as the PD-related genes.

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Development of microarray-based rapid gene analysis system for X-linked adrenoleukodystrophy. -application for genotype-phenotype correlation analysis and search for genes modifying clinical presentations of ALD-. *T. Matsukawa[1], Y. Takahashi[1], O. Onodera[2], N. Shimozawa[3], Y. Suzuki[3], M. Nishizawa[2], J. Goto[1], S. Tsuji[1].* 1) Department of neurology, The University of Tokyo, Tokyo, Japan; 2) Department of neurology, Brain Research Institute, Niigata University, Niigata, Japan; 3) Department of Pediatrics, Gifu University School of Medicine, Gifu, Japan.

Background & Purpose: Adrenoleukodystrophy (ALD) is an X-linked disorder affecting the white matter of the central nervous system occasionally accompanied with adrenal insufficiency. Despite the discovery of the causative gene, *ABCD1*, clear genotype-phenotype correlations have not been established. To investigate the molecular basis for the broad clinical spectrum of ALD, comprehensive gene analyses of not only *ABCD1* but also other candidate genes that modify the clinical presentations would be necessary. To accomplish this aim, we have developed a microarray-based high throughput gene analysis system on *ABCD1* (TKYPD01) and its related gene, *ABCD2* (TKYAD01). The purpose of this study is to validate this system and to explore the possibility of *ABCD2* as the disease modifying gene. Methods: Primers were designed using BLAST search and Smith-Waterman method to avoid amplification of the homologous genes. We applied this PCR system to analyze genomic DNAs from 16 ALD patients with 12 known *ABCD1* mutations and 4 patients with unidentified mutations. All the PCR products were hybridized to DNA microarrays and subjected to scan and analyses. Result: All the known mutations of *ABCD1* were identified by this system. Moreover, four mutations (P84S, H657N, G277R, and W595X) in the patients with adrenomyeloneuropathy. Mutation G277R was found in the patients with adult cerebral form previously reported. A novel SNP was identified in the 5'fUTR of *ABCD2* in a patient with adult cerebral form. Conclusion: Our microarray-based high throughput gene analysis system should be highly useful for mutational analysis of *ABCD1* for patients with ALD, and identifying novel SNPs of related genes to explore SNPs of related genes as the candidates modifying clinical presentations of ALD.

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Importance of open skin biopsy for the diagnosis of CADASIL. *M. Morbin[1], S. Baratta[2], D. Testa[3], D. Pareyson[2], L. Chiapparini[4], O. Bugiani[1], F. Taroni[2].* 1) Div Neuropathology, Istituto Neurologico C Besta, Milan, Italy; 2) Div Biochem & Genet, Istituto Neurologico C Besta, Milan, Italy; 3) Dept Clin Neurosci, Istituto Neurologico C Besta, Milan, Italy; 4) Div Neuroradiol, Istituto Neurologico C Besta, Milan, Italy.

The most appropriate procedure for the diagnosis of CADASIL is still debated. CADASIL is a hereditary cerebrovascular disease that segregates with mutations in the NOTCH3 gene and is characterized by mid-adult onset multiinfarct encephalopathy with headache, multiple stroke and dementia. Deposits of granular osmiophilic material (GOM) in the basal lamina of arterioles are the ultrastructural hallmark of the disease. Although GOM can be identified by skin biopsy, the false-negative rate of this finding is still unknown. Therefore, ultimate diagnosis is based on DNA analysis. Evidence from our and other laboratories indicate that approx. 75% of mutations cluster in exons 3 and 4, which are sequenced routinely to assess the clinical suspect. Negative results raise the problem of mutations elsewhere. Since molecular analysis of the full 33-exon gene can hardly be given as a routine service, complementary methods, such as skin biopsy, should be considered to justify extended DNA analysis. In spite of 100% specificity, sensitivity of skin biopsy in CADASIL has been reported to be as low as 50%, possibly due to the bioptic procedure employed (punch vs. open biopsy). We have carried out open skin biopsies in 9 out of 21 index patients with clinical and MRI findings consistent with the diagnosis of CADASIL. GOM was detected by electron microscopy (EM) in all such patients, who had distinct mutations involving an Arg-to-Cys substitution (2 in ex 3, 3 in ex 4, 1 in ex 2, 1 in ex 8, 1 in ex 11, 1 in ex 19). These results justify to proceed in additional patients as follows. The clinical suspect of CADASIL should be first screened for mutations in NOTCH3 exons 3 and 4; if negative, an open skin biopsy with EM analysis for GOM should be carried out; if this is positive, sequencing of the remaining NOTCH3 exons is strongly recommended. (Partly supported by grant RF2002/160 from the Italian Ministry of Health to FT).

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Mutational Analysis of the *LRRK2* Gene in a Clinic-Based Sample of Patients with Parkinson's Disease. *C.P. Zabetian[1,2], A. Samii[2,3], A.D. Mosley[4], J.W. Roberts[5], B.C. Leis[4], D. Yearout[1,2], W.H. Raskind[6,7,8], A. Griffith[4].* 1) Geriatric Research Education & Clinical Center, VA Puget Sound Health Care System, Seattle, WA; 2) Department of Neurology, University of Washington, Seattle, WA; 3) Parkinsons Disease Research, Education & Clinical Center, VA Puget Sound Health Care System, Seattle, WA; 4) Booth Gardner Parkinson's Care Center, Evergreen Hospital, Kirkland, WA; 5) Virginia Mason Medical Center, Seattle, WA; 6) Department of Medicine, University of Washington, Seattle, WA; 7) Department of Psychiatry & Behavioral Sciences, University of Washington, Seattle, WA; 8) Mental Illness Research Education & Clinical Center, VA Puget Sound Health Care System, Seattle, WA.

BACKGROUND: Recent studies of referral-based samples indicate that a single mutation (G2019S) in exon 41 of the *LRRK2* gene accounts for an unexpectedly large proportion of both familial and sporadic cases of Parkinson's disease (PD). If these data are representative of PD in the general population, then genetic testing for *LRRK2* mutations might be useful in clinical settings. METHODS: We resequenced exons 31, 35, and 41 of the *LRRK2* gene in 371 PD patients consecutively recruited through five movement disorder clinics, and in 281 controls. RESULTS: We found mutations in six (1.6%) PD patients, including two heterozygous for new putative pathogenic variants (R1441H, IVS31+3AG). None of these mutations were observed in the control group. R1441H replaces a highly conserved amino acid, while IVS31+3AG is predicted to disrupt splicing. We also found evidence of substantially reduced penetrance in a family with a previously reported pathogenic mutation (R1441C). CONCLUSIONS: Our findings in a clinic-based PD sample suggest that *LRRK2* mutations are sufficiently common to justify testing in neurological practice. However, more data are needed on the spectrum, frequency, and penetrance of pathogenic variants in the gene. We have now begun to screen the entire *LRRK2* coding region (51 exons) in large numbers of PD patients to address these issues.

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Identification in Drosophila of genetic modifiers of the Tau-induced neurodegeneration. *M. Lecourtois, J. Bou, B. Chaumette, O. Blard, D. Campion, T. Frebourg.* Inserm U614, IFRMP, Faculty of Medicine, 22 boulevard Gambetta, 76183 Rouen, France.

Tauopathies, including Alzheimers disease (AD) and fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), are a group of neurodegenerative disorders characterized by the presence of intraneuronal filamentous inclusions of aberrantly phosphorylated tau. Tau is a neuronal microtubule-associated protein involved in microtubule assembly and stabilization. To investigate the molecular mechanisms responsible for Tau-induced neurodegeneration, we used Drosophila as model of tauopathies. Expression of human Tau in the Drosophila brain recapitulates several features of human tauopathies, including age-dependent neurodegeneration, early death, abnormally phosphorylated and folded Tau. We have performed a deregulation screen to identify genetic modifiers of Tau-induced neurodegeneration. Based on the GAL4-UAS expression system, Tau expression was targeted to the fly eye using the GMR-GAL4 driver line. We have screened 1400 insertion lines containing the P[UAS] transposon elements. This genetic screening allowed us to detect numerous interactors which function is in agreement with the potential biological role of Tau.

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Analysis of ARX gene in X-linked Lissencephaly in an extended pedigree with multiple affected and consanguinity. *A.J. Parsian[1], M. Karim[1], D. Fair[1], M. Elsayed[2], E. Elsobky[2], A. Parsian[1].* 1) Dept Pediatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Medical Genetic Center, Cairo, Egypt.

Lissencephaly is a human neuronal migration disorder associated with abnormal cortical layering leading to a thickened neocortex with mixed agyria and pachygyria or pachygyria alone. X-linked Lissencephaly gene cause classic Lissencephaly in hemizygous males and a milder phenotype known as subcortical band heterotopia in females, sometimes exist in the same family. There are several X-linked loci that affect neuronal migration. Reported mutations in Aristaless-related homeobox gene ARX (Xp21.3) in X-linked Lissencephaly with abnormal genitalia (XLAG) and the phenotype in Arx null mice supported us to screen this gene first in our extended pedigree from Egypt. The pedigree includes two nuclear families with affected children and consanguinity. The male proband (from consanguine parents) has the diagnosis of Lissencephaly, mental retardation and abnormal genitalia. We excluded initially the two known reported mutations in ARX exon 2 by PCR assay and AflIII and HpyCH4V restriction enzyme digestion. We then sequenced the entire ARX coding regions and flanking introns including the splice sites using genomic DNA from affected, carrier, and unaffected pedigree members. We did not detect any mutation in ARX gene in our pedigree, suggesting genetic heterogeneity. We will investigate other X-linked loci for abnormal neuronal migratory disorder such as doublecortin (DCX).

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Chromosome 18qter region is excluded in an extended pedigree with Marinesco-Sjögren syndrome. *J.W. Bracey[1], A.J. Parsian[1], M. Elsayed[2], E. Elsobky[2], A. Parsian[1].* 1) Dept Pediatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Medical Genetic Center, Cairo, Egypt.

Marinesco-Sjögren syndrome (MSS) is a rare, autosomal recessive disorder with several distinguished symptoms. The syndrome is usually characterized by cerebellar ataxia, congenital cataracts, hypotonia, and mild to moderate mental retardation. Other observed symptoms include short stature, dysarthia, additional skeletal deformities, and occasionally hypergonadotrophic hypogonadism. Previous studies in a gypsy family linked a subtype of MSS to the chromosome 18qter along with congenital cataracts-facial dysmorphism-neuropathy (CCFDN) syndrome, indicating these as two overlapping disorders (Merlini et al., 2002). However, more recent genetic linkage mapping found MSS in consanguineous families of Turkish and Norwegian origin to be linked to chromosome 5q31, lacking overlap with CCFDN and indicating the two as genetically distinct disorders (Lagier-Tourenne et al., 2003). In this study, a consanguineous Egyptian pedigree with typical MSS symptoms in affected members in two generations is reported. We screened 12 family members, in three generations, with four microsatellite markers (D18S1122, D18S1141, and D18S1390, D18S70) on chromosome 18qter to determine the linkage to this region. Using homozygosity mapping, none of the four affected pedigree members were homozygous for haplotypes in the region of 18qter. The result of linkage analysis using a recessive model with full penetrance was also negative. Therefore, we excluded the region of chromosome 18qter as a candidate for MSS in our pedigree. Our result also supports the previous linkage finding by Lagier-Tourenne et al., (2003).

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***AHI1* mutations cause both retinal dystrophy and renal cystic disease in Joubert syndrome.** *M.A. Parisi[1], D. Doherty[1], M.L. Eckert[1], D.W. Shaw[2], H. Ozyurek[3], O. Giray[4], A. Al Swaid[5], S. Al Shahwan[5], N. Dohayan[5], E. Bakhsh[6], O.S. Indridason[7], W.B. Dobyns[8], C.L. Bennett[1], P.F. Chance[1], I.A. Glass[1].* 1) Dept Ped/Div Gen & Develop, Children's Hosp & Reg Med Ctr, Seattle, WA; 2) Dept Radiol, Children's Hosp & Reg Med Ctr, Seattle, WA; 3) Dept Ped, Hacettepe Univ, Ankara, Turkey; 4) Dept Ped Gen, Dokuz Eylul Univ, Izmir, Turkey; 5) Dept Paed, Riyadh Armed Forces Hosp, Riyadh, Saudi Arabia; 6) Dept Radiol, Riyadh Armed Forces Hosp, Riyadh, Saudi Arabia; 7) Dept Med, Landspitali-Univ Hosp, Reykjavik, Iceland; 8) Dept Hum Gen, Univ Chicago, Chicago, IL.

Joubert syndrome (JS) is an autosomal recessive disorder characterized by hypotonia, ataxia, mental retardation, altered breathing pattern, abnormal eye movements, and a brain malformation known as the "molar tooth sign" (MTS) on cranial MRI. Four genetic loci have been mapped, with two genes identified (*AHI1* and *NPHP1*). We performed a combination of haplotype analysis and sequencing of *AHI1* in a cohort of 117 JS subjects, and found 15 mutations in 13 families, representing 11% of the group. We identified a variety of novel *AHI1* mutations, including nonsense, missense, splice-site, and insertion mutations, with clustering in the WD40 domains. Eight families with *AHI1* mutations were consanguineous, but no single founder mutation was found. In addition to the MTS, retinal dystrophy was present in 10 of 12 informative families; however, no subjects exhibited other variable features of JS such as polydactyly, colobomas, or liver fibrosis. In contrast to previous studies of *AHI1* mutations, we report two families with siblings who developed renal disease compatible with nephronophthisis (NPH) in their 20s. The same cohort was tested for deletions in *NPHP1*, and two individuals with classic NPH were found to have a homozygous *NPHP1* deletion, representing ~2% of the group. Overall, the contribution of *NPHP1* and *AHI1* mutations is less than 15% in our cohort. Preliminary genotype-phenotype correlations suggest the association of renal impairment, specifically NPH, in those with *NPHP1* deletions. Those with *AHI1* mutations appear to be at risk of developing both retinal dystrophy and progressive kidney disease.

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Homozygous T118M substitution in *PMP22* is associated with severe neuropathy whereas heterozygous mutation can be associated with an HNPP phenotype. *G.M. Saifi[1], M.T. Scavina[2], A. Clark[2], K.M. Krajewski[3], J. Kamholz[3], E. Kolodny[4], K. Szigeti[1], J.R. Lupski[1,5], M.E. Shy[3].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Neurology, duPont Hospital for Children, Wilmington, Delaware; 3) Department of Neurology and Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan; 4) Division of Neurogenetics, NYU School of Medicine, New York; 5) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

PMP22 encodes a tetraspan myelin membrane protein expressed in Schwann cells of the peripheral nervous system. Altered dosage of, and point mutations in, *PMP22* cause the inherited peripheral neuropathies Charcot-Marie-Tooth disease type 1A (CMT1A) and Hereditary Neuropathy with Liability to Pressure Palsies (HNPP). Both conditions are characterized by length dependent limb weakness and sensory loss. The clinical consequences of the amino acid substitution of threonine to methionine at amino 118 in *PMP22* (T118M), however, has been the subject of controversy. Whereas the initial evidence suggested T118M represented a recessive disease causing allele, subsequent findings interpreted this mutation as a benign polymorphism. We identified homozygous T118M mutation in an 11 year old girl with severe progressive distal weakness, diminished temperature and pinprick sensation in a stocking/glove distribution with preserved vibration and proprioception and gait abnormalities whose nerve conduction and needle EMG studies demonstrated progressive length dependent axonal degeneration without demyelination. Her heterozygous T118M parents are both clinically and electrophysiologically normal. Furthermore, we identified three patients heterozygous for the T118M mutation each of whom manifested a clinical neuropathy consistent with HNPP. Our findings support the contention that T118M is a pathogenic partial loss-of-function mutation, but that its clinical expression may depend on the genetic context or background.

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A potential *RAI1* point mutation hotspot in a heptameric C-tract associated with nondeletion Smith-Magenis syndrome. *H. Firth[1], W. Bi[2], G.M. Saifi[2], J.R. Lupski[2,3,4].* 1) Department of Medical Genetics, Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston, TX.

Smith-Magenis syndrome (SMS, MIM182290) is a mental retardation disorder characterized by craniofacial features and neurobehavioral abnormalities. The majority (75-80%) of SMS patients have a heterozygous ~ 4 Mb common deletion in 17p11.2 mediated by non-allelic homologous recombination (NAHR) between SMS-REPs, low-copy repeats flanking the deletion. About 20-25% patients harbor unusual-sized deletions (smaller or larger). Up-to-date, a heterozygous point mutation in the retinoic acid induced 1 gene (*RAI1*) encoding a PHD zinc finger containing transcriptional regulator has been reported in nine phenotypic SMS patients without deletion detectable by FISH. All the mutations are unique, located within exon 3, and de novo, associated with sporadic disease. We report here a novel *RAI1* frameshift mutation in a nondeletion SMS patient. This individual has many SMS features: characteristic craniofacial dysmorphology (midface hypoplasia and broad face), brachydactyly, obesity, and neurobehavioral anomalies including learning disability, speech delay, skin picking, increased pain threshold, and marked sleep disturbance (night time waking, day time sleeping). The mutation is 3102delC, a deletion of a single cytosine in a heptameric C-tract (CCCCCCC), the longest single nucleotide repeat in the *RAI1* coding region. Interestingly, we previously identified a frameshift mutation 3103insC, an insertion of a single cytosine, in the same region. Thus, the heptameric C-tract in *RAI1* is a potential hotspot (2 out of 10 individuals) for the point mutation, likely because of DNA polymerase slippage, in nondeletion SMS patients.

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Mutations in the GABRA1 and EFHC1 genes are rare in autosomal dominant juvenile myoclonic epilepsy. *S. Ma[1], B. Abou-Khalil[1], M.A. Blair[1], A.H. Lagrange[1], J.L. Haines[2,3], P. Hedera[1,2].* 1) Department of Neurology, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.

Juvenile myoclonic epilepsy, accounting for approximately 25% of idiopathic generalized epilepsies, is genetically heterogeneous. The first genes causing autosomal dominant (AD) JME have been only recently identified. A mutation in the alpha-1 subunit of the GABAA receptor has been reported in a single family and 5 mutations have been reported in 6 families in the EFHC1 gene, encoding a protein with an EF-hand, a calcium-binding motif. We have investigated the contribution of these 2 genes to familial JME in our cohort of 35 families. Syndromic classification of JME was based on previously published criteria. We considered kindreds with at least one affected first-degree relative and the evidence of a vertical transmission as definite ADJME, and families with at least one affected second-degree relative as probable ADJME. We included 16 families meeting criteria for definitive ADJME and 19 classified as probable ADJME. None of these families were considered informative enough to analyze candidate loci for JME using linkage analysis. We have systematically screened coding exons of these 2 genes using temperature gradient capillary electrophoresis. Every heteroduplex with an abnormal mobility was sequenced. We did not identify any disease-causing mutations in the GABRA1 and EFHC1 genes. Several coding polymorphisms have been found but the allelic frequency did not differ between controls and affected individuals. Our preliminary data suggests that the majority of familial ADJME is not caused by mutations in the GABRA1 and EFHC1 genes.

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Analysing the function of ALF proteins in the CNS. *K. Davies, E. Bitoun, P.L. Oliver, J. Clark, O. Osborn.* MRC Functional Genetics, Unit, Dept Human Anatomy & Genetics, Univ Oxford, Oxford, United Kingdom.

We have established that AF4 plays a vital role in neuronal survival which would not have been predicted from its known function or from knockout studies. AF4, AF5Q31 and LAF4 are translocated in acute leukaemias and with FMR2, mutated in FRAXE mental handicap, these proteins make up the ALF family of transcription factors. Af4 is mutated in the dominant robotic mouse mutant characterised by ataxia and Purkinje cell loss. The E3 ubiquitin ligase Siah-1a was identified as an interacting protein in the brain and binding assays revealed a significant reduction in the affinity of Siah-1a with robotic Af4 compared to wild-type. This correlates with an almost complete abolition of mutant Af4 degradation by Siah-1a, leading to an accumulation of the mutant protein in the robotic mouse. Fmr2 binds Siah proteins in a similar manner, suggesting that this common regulatory mechanism regulates the levels of ALF proteins. We have also investigated the influence of the mutation on the transcriptional activation properties of these proteins and analysed its functional significance in neuronal cell cultures. The robotic mouse therefore provides a unique opportunity to understand how ALF proteins play a role in disorders as diverse as leukaemia, mental retardation and neurodegeneration.

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A mutation in the cathepsin D gene (*CTSD*) in American Bulldogs with neuronal ceroid lipofuscinosis. *G.S. Johnson[1], T. Awano[1], J.F. Taylor[2], D.P. O'Brien[3], S. Khan[1], J. Evans[4], I. Sohar[5], P. Lobel[5], M.L. Katz[1,6].* 1) Veterinary Pathobiology, University of Missouri, Columbia; 2) Animal Sciences, University of Missouri, Columbia; 3) Veterinary Medicine and Surgery, University of Missouri, Columbia; 4) Veterinary Neurological Center, Las Vegas, NV; 5) Center for Advanced Biotechnology and Medicine, University of Medicine and Dentistry of New Jersey, Piscataway; 6) Mason Eye Institute, University of Missouri, Columbia.

We examined DNA, brains and eyes from American Bulldogs with signs of neurodegeneration that appeared before two years of age and progressed slowly leading to death by 4 to 6 years of age. Autofluorescent cytoplasmic inclusions were observed throughout the brains, in retinal ganglion cells and along the outer limiting membrane (OLM) of the retinas, establishing a diagnosis of neuronal ceroid lipofuscinosis (NCL). Electron microscopy revealed that the inclusions consisted of coarsely granular matrices surrounding well-delineated spherical structures and that the inclusions along the OLM were within photoreceptor cells, primarily cones. Affected American Bulldogs were homozygous for the A allele of a G to A missense mutation in *CTSD* which predicts the conversion of methionine-199 to isoleucine. Only the G allele was detected in DNA samples from 131 randomly selected dogs of 108 other breeds; however, the A allele had a frequency of 0.26 among the 123 genotyped American Bulldogs. Pedigree analysis indicated a probability of less than 10^{-12} that another locus unlinked to *CTSD* was the true NCL-causing mutation. Enzyme activity measurements on brain extracts revealed that cathepsin D activity in affected dogs was 34% of that found in unaffected controls while 15 other lysosomal enzyme activities were either unchanged or increased. Previously described *CTSD* knockout mice exhibited NCL with viseral complications and sheep lacking cathepsin D enzyme activity were born with a grossly underdeveloped CNS. In comparison, the clinical course of NCL in the American Bulldogs was less severe with a later onset, thus, more closely resembling that of many of the human NCLs. This suggests that *CTSD* should be considered a candidate locus for human NCLs of unknown cause.

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A novel homozygous recessive mutation in PINK1 gene associated with Early-Onset Parkinsons Disease in a Saudi family. *M.A. Chishti[1], P. Carroll[2], M. Ahmed[1], A. Loualich[1], E. Rogaeva[3], S. Bohlega[4].* 1) Transgenic Laboratory, CMD, KFSH&RC, Riyadh,Saudi Arabia; 2) Aragene, KFSH&RC, Riyadh, Saudi Arabia; 3) CRND, Department of Medicine, UofT, Toronto, ON, Canada; 4) Department of Neurosciences, KFSH&RC, Riyadh, Saudi Arabia.

Parkinsons Disease (PD) is a neurodegenerative disorder that is characterized by progressive dysfunction of movement due to the predominant degeneration of dopaminergic neurons in the basal ganglia. Although familial PD represents less than 10% of all cases, parkinsonism-causing mutations have been defined in at least five different genes: SNCA, Parkin, DJ-1, LRRK2 and PINK1. Mutations in the PINK1 gene have been associated with the hereditary early-onset form of PD. PINK1 encodes a 581 amino acid protein with two predicted domains: a mitochondrial targeting motif and a highly conserved protein kinase domain (residues 156 to 509) that shows a high degree of homology to the serine/threonine kinases of the Ca2+/calmodulin family. In a study recently commenced, the entire coding region of the PINK1 gene (8 exons with intron/exon boundaries) has been screened in a total of 5 consanguineous Saudi families with a clinical diagnosis of early onset PD (less than 50 years of age). In one of five families screened, a novel missense homozygous mutation was found in exon 4, nucleotide 1032 CT (GenBankAB053323) resulting in an amino acid change from threonine to methionine at residue 313 (T313M). This family consists of eleven siblings resulting from a first cousin marriage. To date three of the eleven siblings demonstrate clinical manifestations of early-onset PD. The one affected female sibling began to experience tremulous movements of her hands at age 29 years, the two affected male siblings both report onset of symptoms at the age of 32 years. The T313M amino acid change occurs in a region of the protein kinase domain that is conserved across six species surveyed. It is reported that wild type PINK1 may protect neurons from stress-induced mitochondrial dysfunction and stress-induced apoptosis and it may be this effect that is abrogated by the T313M mutation reported here, resulting in early-onset PD.

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A mutation in carbonic anhydrase related protein 8 causes ataxia in the mouse waddles mutant. *A. Nystuen.*
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The waddles (*wdl*) mutant is a mouse model for human ataxia. *Wdl* is a recessive, non-lethal mutant characterized by a non-progressive, ataxic gait. The mutation was mapped to a 6 megabase region on chromosome 4. Rather than further genetic fine mapping, I used a comprehensive informatics approach to identify functionally relevant candidate genes. Mutational analysis identified a 19bp deletion in exon 8 of the carbonic anhydrase related protein 8 (*Car8*) gene. This deletion was not detected in a survey of common and wild mouse strains including the strain of origin. *Car8* functions in the inositol pathway by interacting with 1,4,5-trisphophate receptor type I (IP3R1) to regulate intracellular Ca^{2+} in Purkinje cells. The mutation results in a frame shift causing a nonsense codon 21 amino acids downstream and removes part of the protein necessary for interaction with IP3R1. The *wdl* mutant further demonstrates the central importance of the inositol second messenger system in the Purkinje cell. Phenotypic and histological analysis showed that *wdl* has a relatively mild phenotype when compared to other mutants in this pathway. Thus, *wdl* becomes an important model for pure ataxic disorders. In fact, an ataxic patient with circulating antibodies to CAR8 has been described. Additionally, *Car8* is over-expressed at tumor invasion fronts of certain cancers, suggesting that this mutant may be a useful model to study the process of invasion.

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Role of CSTB in neuronal survival: Implications to disease mechanisms in progressive myoclonus epilepsy. *S. Tegelberg[1], T. Joensuu[1], K. Alakurtti[1], E. Weber[2], O. Kopra[3], A.-E. Lehesjoki[1].* 1) Folkhälsan Institute of Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland; 2) Institute of Physiological Chemistry, Martin Luther University Halle-Wittenberg, Halle, Germany; 3) Deptartment of Molecular Medicine, National Public Health Institute and Neuroscience Center, University of Helsinki, Finland.

Loss-of-function mutations in the gene encoding cystatin B (CSTB), a cysteine protease inhibitor, underlie progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1), an autosomal recessive disorder. EPM1 is characterized by onset at 6-15 years of age, stimulus-sensitive myoclonus, tonic-clonic seizures, characteristic EEG-findings, ataxia and a progressive course. *Cstb*-deficient mice develop a phenotype resembling that of human EPM1 patients including progressive ataxia and myoclonic seizures. The mice show severe cerebellar granule cell apoptosis as well as neuronal atrophy, apoptosis and gliosis in the cerebrum, suggesting that CSTB has a role in preventing neuronal apoptosis in specific cell types and in maintaining normal neuronal structure. In order to understand the neuronal function of CSTB, we have initiated studies on its neuronal localization, as well as on its role in neuronal survival, utilizing primary neuronal cultures and tissues of *Cstb*-deficient and wild-type mice. As the cerebellar granule cells in *Cstb*-deficient mice die apoptotically, we aim to investigate the oxidative and non-oxidative conditions under which CSTB is required for neuronal survival by using brain tissue and neurons derived from *Cstb*-deficient mice. Towards the neuronal localization of CSTB, we have produced novel monoclonal antibodies for mouse CSTB that we are currently characterizing. Initial results from experiments using embryonic cortical neurons revealed no differences in oxidative or non-oxidative stress responses between wild-type and *Cstb*-deficient mice. Further experiments in cerebellar granule cells are ongoing. We are also investigating the apoptotic cell death and the possible neuronal re-entry to cell cycle in *Cstb*-/- neurons on tissue level in order to monitor the spatial and temporal pathology in the early stages of the disease.

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Functional analysis of the Cacna2d2 calcium channel gene using transcriptional profiling in the ducky mouse mutant: a model of epilepsy and ataxia.*Cacna2d2*. *M. Rees[1], T. Wilkinson[1], A.C. Dolphin[2].* 1) Pediatrics, UCL Medical Sch, London, United Kingdom; 2) Pharmacology, UCL, London, United Kingdom.

The mouse mutant ducky (du)is characterised by spike-wave seizures and cerebellar ataxia. Loss of function mutations in the Cacna2d2 gene were found to underlie the phenotype in du strain and its allele, du2J. Cacna2d2 encodes the 2-2 voltage-dependent calcium channel auxiliary subunit, which is highly glycosylated and largely extracellular, and is strongly expressed in cerebellar Purkinje cells. We have shown that homozygous du/du mice have abnormalities in their Purkinje cell dendritic tree and electrophysiological recordings from dissociated Purkinje cells showed a 40% reduction in whole cell calcium current. In addition, co-expression of the truncated 2-2 protein predicted by the original du mutation, with the Cav2.1/4 channel combination in vitro leads to a reduction in current density To aid elucidation of additional function(s) of 2-2 we have performed Affymetrix microarray analysis to analyse transcriptional differences between du2J/du2J and wild type cerebellum at three different stages of development (perinatal [P0], postnatal day 10 [P10] and around weaning [P21]). We have focused on the cerebellum as this is the region showing highest expression of 2-2. We expect that those transcripts identified as up- or down-regulated, will act up- or downstream of 2-2, either via the calcium channel as a whole, or directly though the 2-2 subunit and may provide further insight into the function of the 2-2 subunit. Results showed a marked reduction in Cacna2d2 message at all ages, implying nonsense mediated decay. The other genes identified as having altered expression at P0 have no overlap with those identified at P21, implying a dual role for 2-2 in development. To support this, no differences were seen at P10. At P0, genes showing changes in expression are largely uncharacterised ESTs, suggesting a novel pathway. At P21, there was reduced expression of a number of genes including an extracellular matrix protein and an angiotensin receptor 1-like transcript. All gene expression changes were confirmed by quantitative PCR.

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VCP mutations in two kindreds with prominent frontotemporal dementia: clinical features and neuropathological examination. *L. Marechal[1], A. Laquerrière[2], C. Dumanchin[1], C. Duyckaerts[3], J. Bou[1], D. Hannequin[1], T. Frébourg[1], D. Campion[1].* 1) Inserm U614, IFRMP, Faculty of Medicine, Rouen, France; 2) Department of Pathology, Rouen University Hospital, France; 3) Laboratory of Neuropathology Escourolle, La Salpétrière Hospital, Paris, France.

Inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD) is a unique autosomal dominant disorder (OMIM 167320). The gene was mapped to chromosome 9p13.3-p12 and missense mutations within the gene encoding valosin-containing protein (VCP) have recently been identified. VCP/P97, an abundant homohexameric intracellular protein, is an AAA class ATPase able to bind directly to polyubiquinated proteins. It can exert a variety of cellular functions through interaction with specific adaptators, e.g. p47, UFD1 and Np14. VCP is strongly involved in the ubiquitin-proteasome dependent degradation of cytosolic proteins and in the retrotranslocation of missfolded proteins through the ER associated degradation pathway. We describe the clinical features of two large kindreds in which VCP R93C and R155C missense mutations segregate. We show that dementia concerned 100 % of affected subjects in family I and 55% in family II (as compared with 30% in average in previously described families). Dementia was the initial symptom in 50% of affected cases in family I and 11% in family II. By contrast, muscle weakness and PBD were much inconstant clinical features. We also report detailed neuropathological data from three cases provisionally diagnosed as "dementia lacking distinct histopathological features" after routine examination. Further examination revealed additional features, consistent with the hypothesis that the VCP R155C mutation disrupts normal VCP function, leading to diffuse accumulation of ubiquinated proteins within the cells. Ubiquinated proteins did not form aggresomes and the proteasomal function by itself remained intact. In past years, impairment of Ubiquitin-dependant proteolysis in neurons has emerged as the prime suspect in causing neurodegenerative diseases. Thus, IBMPFD linked VCP mutations define a novel class of alteration of the Ubiquitin-proteasome system.

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Low frequency of PDCD10 mutations in a panel of CCM3 probands: potential implications for a fourth CCM locus. *C.L. Liquori[1], M.J. Berg[2], F. Squitieri[3], M. Ottenbacher[1], M. Sorlie[4], T.P. Leedom[1], M. Cannella[3], V. Maglione[3], L. Ptacek[5], E.J. Johnson[4], D.A. Marchuk[1].* 1) Dept of Molecular Genetics and Microbiology, Duke Univ Medical Center, Durham, NC; 2) Strong Epilepsy Center, Dept of Neurology, Univ of Rochester Medical Center, Rochester, NY; 3) Neurogenetics Unit, Istituto di Ricovero e Cura a Carattere Scientifico, Neuromed, Pozzilli, Italy; 4) Molecular Diagnostics and Biobanking, Prevention Genetics, Marchfield, WI; 5) Dept of Neurology, Univ of California San Francisco, San Francisco, CA.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can result in a variety of neurological disabilities, including stroke and seizures. Linkage analyses using autosomal dominant families manifesting CCMs have identified three different causative loci on chromosomes 7q21.2 (*CCM1*), 7p13 (*CCM2*), and 3q25.2-q27 (*CCM3*). Multipoint linkage data from a large number of families suggests that CCM1 accounts for 40% of all familial CCM cases, CCM2 accounts for 20%, and CCM3 accounts for 40%. Mutations in the gene *Krit1* are responsible for CCM1, mutations in the gene *MGC4607* are responsible for CCM2, and mutations in the gene *PDCD10* were recently reported to be responsible for CCM3. We report here that sequence analysis of *PDCD10* in a panel of 28 probands lacking *Krit1* and *MGC4607* mutations revealed only three mutations. The frequency of identified mutations in the *PDCD10* gene was surprisingly low at only 11%, especially given that this panel was heavily biased towards non-CCM1, non-CCM2 probands. These data are in stark contrast with the linkage data, which suggested that 40% of inherited cases would be due to mutations in this gene. Interestingly, when examining the haplotypes of previously published CCM3 families, we found a distinct recombination event in one of the largest CCM3 families that excludes the *PDCD10* gene. Although there are many potential explanations for this observation, when combined with the apparent under-representation of causative CCM mutations in *PDCD10*, this recombination event in a CCM3-linked family suggests that there may be an additional CCM gene in the same chromosomal region.

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Analysis of IL-4, MxA, and IRF-1 Genes in Filipino Patients with Subacute Sclerosing Panencephalitis. *C.L.T. Silao[1,2], J.R. Pipo-Deveza[2], K. Kusuhara[3], A.M. Salonga[2], M.B. Lukban[2], B.C. Sanchez[2], R. Kira[3], H. Torisu[3], T. Hara[3].* 1) Institute of Human Genetics, National Institutes of Health Philippines, University of the Philippines Manila; 2) Department of Pediatrics, University of the Philippines - Philippine General Hospital; 3) Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Kyushu, Japan.

Subacute Sclerosing Panencephalitis (SSPE) is a chronic and debilitating disease of the central nervous system caused by a latent measles infection. Although reported to be rare globally, this may not be so in a country like the Philippines were the prevalence of measles remains high. Three candidate genes, IL-4, MxA, and IRF-1 were shown to be associated among Japanese patients with SSPE. These genes have been suggested to play a role in the establishment of persistent viral infection in the central nervous system. To determine if there is an association of the IL-4, MxA, and IRF-1 promoter genes to SSPE susceptibility, a total of 180 samples (60 Filipino SSPE patients and 120 healthy control subjects) were included in the study. Demographic characteristics of the patients were recorded using an SSPE registry form. Single nucleotide polymorphisms at -590C/T IL-4 and -88G/T MxA promoter regions were screened using the PCR-RFLP method. Genotyping was done for the IRF-1 gene and association studies were performed between genotypes and SSPE. Our data showed that there is male predominance among the SSPE patients with a mean age at the time of diagnosis of 11.3 years old and an age range of 3-24 years old. Fifty-two percent had measles before one year of age. The TT genotype of MxA as well as the CT genotype of IL-4 were more frequently seen in SSPE patients as compared to the control subjects. The values though, did not reach statistical significance. IRF-1 gene analysis did not differ among the two groups. Our study demonstrated that there is no significant association of IL-4, MxA and IRF-1 genes among Filipino SSPE patients. Further studies are presently being conducted by our group by way of screening other candidate genes that may confer genetic susceptibility to SSPE.

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Mechanisms of intranuclear rod formation in human disease. *K.N. North[1,2], A. Domazetovska[1,2], B. Ilkovski[2], M. Ghodoussi[3], E.C. Hardeman[3], S.T. Cooper[1,2].* 1) University of Sydney, Australia; 2) Institute for Neuromuscular Research, The Childrens Hospital at Westmead, Sydney, Australia; 3) Childrens Medical Research Institute, Sydney, Australia.

The -actinins are actin-binding proteins involved in establishing and maintaining cell structure. Abnormal accumulation of -actinin inside the nucleus is a recognized pathologic feature of some inherited myopathies. Accumulation of actin also occurs in the nucleus due to oxidative stress and is a feature of some neurodegenerative disorders. The mechanisms underlying intranuclear rod formation and their effects on cell function are unknown. Mutation at Val163 in -skeletal actin (*ACTA1*) result in muscle weakness and the formation of rods exclusively within the nuclei of muscle cells. These rods are abundant in -actinin-2 and filamentous actin, providing us with a model in which to study the development of abnormal intranuclear inclusions as part of a disease process.

Here we demonstrate for the first time that -actinin is normally found in the nucleus of a variety of cells and its export from the nucleus is sensitive to the nuclear export inhibitor leptomycin B. By live-cell imaging, we show that intranuclear aggregates due to mutations at Val163 in *ACTA1* form inside the nucleus and are dynamic structures. In the presence of mutant actin, the intranuclear inclusions form in muscle and non-muscle cells and contain both muscle and non-muscle -actinins. We then induced the formation of intranuclear inclusions rods by exposing both muscle and non-muscle cells to cytochalasin D. These intranuclear rods contain both wild type actin and -actinin, suggesting that a common mechanism is involved in their formation. We also demonstrate that the trapping of -actinin into intranuclear aggregates has an adverse effect on cell viability and division. These findings provide insight into the pathogenesis of myopathies and neurodegenerative disorders associated with abnormal intranuclear structures.

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ER Quality Control as a Check Point for the Fate of Peripheral Myelin Protein 22. *T. Yang, W. Orfali, X. Chen, R.N. Sifers, G.J. Snipes.* Department of Pathology, Baylor College of Medicine, Houston, TX.

Missense mutations in Peripheral Myelin Protein 22 are responsible for a subset of Charcot-Marie-Tooth disease patients. The majority of PMP22 missense mutations are dominant gain of function mutations associated with retention of the mutant protein and degradation from the endoplasmic reticulum (ER). We previously demonstrated the dominantly inherited Pmp22L16P mutation, which causes hereditary nerve disease in humans and is modeled in the TremblerJ (Tr-J) mouse, is retained in the ER in association with the ER chaperone, calnexin (CNX). We have extended our study of Pmp22L16P to additional mechanisms associated with glycoprotein ER associated degradation (GERAD). Pulse-chase studies of Pmp22wt and Pmp22L16P in HEK293 cells immunoprecipitated with anti-CNX confirm the prolonged association of CNX with the mutant Pmp22 protein as compared to wild-type. Treatment with lactacysin, a proteasome inhibitor, almost completely abolished the degradation of newly-synthesized Pmp22L16P, but had not significant effect on the degradation of Pmp22wt. Both EDEM and ER manI have been shown to affect the degradation of several GERAD substrates. We found that overexpression of EDEM attenuated the association of Pmp22L16P with CNX, but had little effect on the overall rate of degradation of newly-synthesized Pmp22L16P. Inhibition of ER ManI with the alkaloid kifunensin, on the other hand, attenuated the degradation of Pmp22wt and Pmp22Tr-J, whereas overexpression of ER ManI accelerated the degradation of both wild-type and mutant Pmp22. Interestingly, we found that addition of a myc epitope tag to Pmp22 prolonged their half-lives relative to their untagged counterparts, suggesting the need for caution when interpreting the GERAD mechanisms in the degradation of myc-tagged Pmp22. In summary, the findings from untagged Pmp22 confirm that Pmp22wt and Pmp22L16P are substrates for GERAD with markedly different rates of degradation. Pmp22L16P is degraded predominantly via the proteasome, but the primary disposal mechanism for newly-synthesized Pmp22 (85% of which is rapidly degraded) is unknown. *This research is supported by the Muscular Dystrophy Association.

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Interaction of huntingtin associated protein-1 with kinesin light chain: implication for vesicular transport in neurons. *J.R. McGuire, S.H. Li, X.J. Li.* Department of Human Genetics, Emory University, Atlanta, GA.

Huntingtin associated protein 1 (HAP1) was first identified in a yeast-2-hybrid screen using huntingtin (htt) as bait. Unlike htt that is ubiquitously expressed in the body and brain, HAP1 is enriched in brain and is abundantly expressed in the hypothalamus. Consistent with the critical role of HAP1 in neuronal function, HAP1 knockout mice show a postnatal lethality due to a possible feeding defect and neuronal dysfunction in the hypothalamus. HAP1 has been shown to interact with dynactin P150, a subunit of the dynein complex that drives microtubule-dependent retrograde transport in axons. Using the yeast-2-hybrid screen, we found that HAP1 also interacts with the light chain subunit of the kinesin complex, an anterograde motor. This interaction has been confirmed by in vitro binding assays, immunocytochemical colocalization, and immunoprecipitation from PC12 cell and mouse brain lysates. The interaction also appears to increase in strength upon NGF stimulation of PC12 cells. To understand the functional consequence of this interaction, microtubule binding assays and vesicular transport assays have been performed. These experiments suggest that HAP1 binding to microtubules is mediated by the kinesin complex and that HAP1 is involved in kinesin-mediated vesicular transport.

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Haplotype analysis in German SCA17 families. *P. Bauer[1], J. Tomiuk[1], C. Bauer[1], A. Rolfs[2], L. Schoels[3], O. Riess[1].* 1) Dept Medical Genetics, Univ Tuebingen, Tuebingen, Germany; 2) Dept Neurology, Univ Rostock, Rostock, Germany; 3) Dept Neurology & Hertie-Institute of Brain Research, Univ Tuebingen, Tuebingen, Germany.

SCA17 is a progressive neurodegenerative disease leading to cerebellar ataxia and dementia. Several accessorial symptoms such as Parkinsonism, dystonia, and psychiatric disturbances commonly aggravate to disease course. Genetically, a CAG/CAA expansion in the TATA binding protein (TBP) is expanded in SCA17 patients, leading to an expanded polyglutamine chain in this ubiquitously expressed transcription factor. SCA17 is rare among the dominant spinocerebellar ataxias (ADCA). Overall less than 1% of all ADCA families do account for SCA17 mutations. In Germany, several SCA17 families have been identified. In order to assess possible founder effects, we performed a haplotype analysis with markers telomeric of the SCA17/TBP gene on chromosome 6q27 (D6S1585, D6S281, D6S446, D6S1590) and sequenced the repeat structure in all patients. Overall, five German SCA17 families have been investigated. In four families SCA17 haplotype phases could be determined unanimously. In two families from Mecklenburg-Vorpommern (MV) with rather cerebellar pronounced phenotypes the closest markers (D6S1590 and D6S446, haplotype for the pathological chromosome 1-3) displayed an identical haplotype. A large third family from MV with Huntingtons disease-like phenotypes had a different haplotype (1-1). A fourth family from MV could not be assigned unanimously, while the fifth family originating from western Germany showed to have an independent haplotype (1-2). To conclude, haplotype analysis in German SCA17 families does argue for at least three different possible founder mutations. Even those four families clustering within the county MV did show at least two different haplotypes. Additional epidemiological studies are needed in order to clarify dynamics in the TBP CAG/CAA repeat expansion.

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Function of a Neuron-specific Isoform of the *TAF1* Gene. *S. Ando, S. Makino, G. Tamiya.* Department of Neurology, Tokushima Univ Sch Medicine, tokushima, Japan.

We previously found a neuron-specific isoform of the *TAF1* (TATA-binding protein-associated factor 1) gene, which is the disease causative gene of X-linked recessive dystonia-parkinsonism (XDP; OMIM#314250). The *TAF1* gene encodes the largest component of the TFIID complex involved in RNA polymerase II-mediated expression of genes. The neuron-specific isoform of the *TAF1* gene may have an essential role in neuronal survival in the striatum through transcriptional regulation of many neuron-specific genes. To investigate the detailed function of the neuron-specific isoform of the *TAF1* gene, we performed knockdown of the neuron-specific isoform using a specific siRNA in the human dopaminergic neuroblastoma cell line SH-SY5Y. The siRNA significantly reduced mRNA of the neuron-specific isoform to approximately 1/6 of that in the negative control siRNA with no induction of the interferon response. We subsequently performed microarray analysis for the knockdown cells using an Affymetrix Human Genome Focus Array representing 8,793 annotated genes. By these in vitro experiments, we demonstrated that the *TAF1* gene regulates the neuron-specific transcriptions of genes by the neuron-specific isoform.

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Mutational Mapping of the *NF2* Gene: A Meta-Analysis of Published Data and Diagnostic Laboratory Findings.

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A number of single- and multiple-tumor phenotypes have been linked to the inactivation of the *NF2* tumor suppressor gene. To facilitate structural understanding of this gene across phenotypes, we compiled all published alterations since 1993 (N = 689) from 64 publications along with 49 unpublished alterations from our lab into a set of databases encompassing constitutional and somatic events. Somatic data were subdivided into the more common single sporadic tumor phenotype (N=336 including meningiomas and schwannomas among others) and multiple tumor syndromes. We conducted a meta-analysis of these alterations to assess the overall mutational spectrum and compare spectra across phenotypic subgroups. Alongside this dataset we also studied *NF2* alterations (N=125) found since 1994 by the Massachusetts General Hospital Neurogenetics DNA Diagnostic Lab to determine whether this clinical facility processes a representative cross-section of the mutational spectrum. Within the published dataset, we found a dramatic difference in alteration type between constitutional events and somatic events from single tumors, with somatic heavily skewed towards frameshift changes, and constitutional tending towards nonsense and splice-site ($P<0.001$). We also found a statistically significant difference in both mutation type and exon distribution in somatic events from sporadic schwannomas (N=155) and meningiomas (N=150), with the former overrepresented in frameshift mutations and the latter favoring nonsense changes ($P<0.01$). We did not observe a statistically significant difference in mutation type or exon distribution between published constitutional events and those found by the DNA Diagnostic Lab, suggesting a representative sample of tissue submitted for testing. Overall, this meta-analysis provides evidence for a fundamentally different genetic profile between constitutional and somatic events in the *NF2* gene, and among somatic events causing sporadic schwannomas versus those associated with sporadic meningiomas.

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Deficient Glomulin Expression Disrupts Vascular Smooth Muscle Cell Differentiation and Causes Glomuvenous Malformations. *B.A.S. McIntyre[1], L.M. Boon[1,2], V.S. Vassilev[3], P.B. Brouillard[1], J.B. Mulliken[4], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute & University of Louvian Medical School, Brussels, Belgium; 2) Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires St Luc, Brussels, Belgium; 3) Cell Biology Unit, Christian de Duve Institute of Cellular Pathology & University of Louvain Medical School, Brussels, Belgium; 4) Vascular Anomalies Center, Division of Plastic Surgery, Childrens Hospital and Harvard Medical School, Boston, MA, USA.

Glomulin (GLMN) was recently identified in our laboratory as the gene mutated in glomuvenous malformation (GVM). These localized cutaneous anomalies are characterized histologically by enlarged venous channels with a variable number of normal-to-polygonal mural cells termed "glomus" cells (Boon et al. 2004).

Most GLMN mutations cause premature stop codons, probably resulting in non-sense mediated mRNA decay. Demonstration of a second-hit mutation in one GVM lesion suggested paradominant inheritance (Brouillard et al. 2002). GLMN is specifically expressed in vascular smooth muscle cells (vSMC) during murine development (McIntyre et al. 2004). We hypothesize that GLMN mutations alter differentiation of vSMCs due to a complete, localized lack of GLMN expression. Thus, we tested GVMs for the presence of GLMN mRNA using RNA ISH and examined the profile of other vSMC differentiation markers.

We demonstrate that during murine development, GLMN expression begins in vSMCs after desmin, h-caldesmon (h-CD) and smooth muscle myosin heavy chain (SMMHC), but before expression of smoothelin-b (SMTN-B). We also show that glomus cells in GVM do not express GLMN or SMTN-B (2 late vSMC markers), although earlier markers, SMMHC and h-CD, are detected. Expression of all of these markers was observed in vSMCs of venous malformations. We conclude that glomus cells have been halted in their differentiation due to a complete lack of GLMN giving further evidence for paradominant inheritance of GVM. (www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be).

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Defining the clinical phenotype of deletion and duplication 1q21.1 syndromes. *M.J. Somerville, M. Lilley, O.J. Lehmann, M. Hicks, J. Christiansen, R. Tomaszewski, K.A. Sprysak, B.G. Elyas, S.M. Haase, N.J. Leonard, L.M. Vicen-Wyhony.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

A chromosome 1q21.1 multigene deletion syndrome has recently been identified in association with cardiac defects. This condition results in hemizygosity for several genes including GJA5 (connexin40) that is predicted to lead to the congenital heart disease (CHD) observed in these cases. Another gap junction protein (GJA8; connexin50) also deleted in these cases, is primarily expressed in the lens. Missense mutations in GJA8 have been shown to cause pulverulent zonular cataracts. We screened 731 unrelated CHD cases for deletions or duplications of the 1q21.1 region. Dosage was determined by real-time quantitative PCR amplification of ACPL1, GJA5, and GJA8 genes. Deletion breakpoints and parent of origin were determined using microsatellites spanning 1q21.1. In total, 4 cases (4/731) carried deletions and 1 case (1/731) a duplication of the 1q21.1 critical region, which were absent from 520 control cases. All deletion carriers were males with obstruction of the aortic arch. The duplication case was a female with tetralogy of Fallot. Two of the deletions were maternally derived the other one tested was de novo and of paternal origin. The duplication was paternally derived. Although the two mothers with 1q21.1 deletions and the father with a 1q21.1 duplication had no detectable heart defects, all three had lens opacities upon ophthalmic examination. The deletion carriers exhibited congenital opacities of the embryonic lens nucleus while the duplication carrier had small peripheral punctate lens opacities. All the opacities were sub-clinical with no appreciable effect on vision. In summary, we observed altered dosage of chromosome 1q21.1 in 5 out of 731 (0.7%) CHD cases. We hypothesize that altered dosage of the 1q21.1 region is associated with aortic arch defects and lens opacities, that penetrance of the heart defects is sex-dependent, but that sub-clinical lens defects are fully penetrant. Since there are no other consistent phenotypic features of these syndromes, screening individuals for lens opacities may provide a useful phenotypic marker to guide molecular testing.

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ANKRD3, a candidate gene for involvement in Down syndrome heart defects. *R. Lyle[1,2], C. Burgi[1], S. Gagos[1], C. Gehrig[1], S. Nef[1], S. Dahoun[1], S.E. Antonarakis[1].* 1) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 2) Institute of Public Health, Oslo, Norway.

Human autosomal aneuploides are very common but only trisomy for human chromosome 21 (Down syndrome, DS) normally survives beyond the early postnatal period. In addition to the mental retardation and facial characteristics, there are many other phenotypes associated with DS. Notably, 40-45% of DS individuals have congenital heart disease (CHD), generally atrioventricular septal defects (AVSD) or ventricular septal defects (VSD). Since the genes on the triplicated chromosome 21 (Hsa21) are normal, DS is considered to be a problem of gene-dosage. However it is not known which genes, or groups of genes, lead to the specific DS phenotypes. In previous work using the Ts65Dn mouse model of DS, we showed that the gene Ankrd3 was overexpressed ~4-fold in adult mouse heart but only ~1.5-fold in five other tissues. This large overexpression, and the fact that ANKRD3 maps within the putative DS heart critical region, suggests that it may be involved in DS heart defects. To study this possibilty we considered the following questions. Is ANKRD3 overexpressed in human DS fetal heart? Is ANKRD3 expressed at the appropriate time and place in heart development? First we examined the expression of ANKRD3 by qPCR in monozygotic twins discordant for DS and found that it is expressed 4.2-fold higher in the DS compared to euploid heart. This result was confirmed in other fetal hearts (3 DS, 3 normal), with DS/N ratio of 3-5 fold. We next studied the expression pattern of Ankrd3 in E11.5 and E13.5 mouse embryos by in situ hybridization. Ankrd3 is expressed in the developing mouse heart, and most strikingly there is strong expression in the developing heart atrioventricular septum at E11.5. ANKRD3 is therefore a good candidate for involvement in the heart defects most commonly seen in DS. We have also used Affymetrix arrays to study transcriptome changes in both mouse Ts65Dn and human DS heart, and have identified alterations in expression of several genes known to be important for correct heart function.

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Phenotypic spectrum and pathogenesis of Loeys-Dietz aortic aneurysm syndrome. *B. Loeys[1], J. Chen[1], U. Schwarze[2], J. De Backer[4], A. Braverman[3], N. Mc Donnell[5], P. Coucke[4], T. Holm[1], G. Thomas[1], C. Francomano[5], P. Byers[2], A. De Paepe[4], H. Dietz[1,6].* 1) Johns Hopkins Univ, Baltimore; 2) Univ of Washington, Seattle; 3) Wash Univ, St. Louis; 4) Ghent Univ, Ghent; 5) NIH/NIA, Baltimore; 6) HHMI.

A novel autosomal dominant syndrome characterized by the triad of hypertelorism, bifid uvula/cleft palate, and arterial tortuosity with aortic aneurysm/dissection was previously described (Loeys-Dietz syndrome (LDS) MIM#609192). While some individuals show overlap with Marfan syndrome (MFS), none satisfied diagnostic criteria. So far, we have identified 27 mutations in either the type I or type II transforming growth factor beta receptor(TGFBR1/2) in typical LDS patients. In view of some phenotypic overlap between LDS and the vascular form of Ehlers-Danlos syndrome (EDS), we screened an additional cohort of vascular EDS patients with normal type III collagen studies. Their phenotype was characterized by typical skin findings, uterine rupture and arterial aneursym/dissections within the cerebral, thoracic and abdominal circulations. We identified heterozygous TGFBR mutations in 12 families, thus extending the clinical continuum of LDS. A clustering of mutations in TGFBR2-exon 5 (encoding the middle part of the kinase domain) was found in EDS-like patients, whereas typical LDS patients had mutations at both ends of the kinase domain. Despite evidence that receptors derived from mutant alleles cannot support TGF signaling, we have shown that heterozygous patient-derived fibroblasts retain appropriate responsiveness to TGF-ligand. Furthermore, tissues derived from affected individuals show increased output from TGF-responsive genes (eg CTGF) and increased pSmad2, indicative of increased TGF signaling. The study of VSMCs derived from patient aortas demonstrates inappropriate overactivation of the p38 and ERK1/2 cascades, both at baseline and in responses to TGF. In parallel, the production of CTGF by VSMCs is upregulated, thus correlating in vitro with in vivo observations. A pathogenetic model is proposed in which activation of these cascades is both a consequence of and a contributor to dysregulated TGF signaling.

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Mutation Analysis of TBX5 in Families with Holt-Oram Syndrome: Viability of Composite Heterozygous Mutation in TBX5. *T. Huang[1,2], A. Boyadjian[1], X. Xie[1], J.G. Seidman[3], C.E. Seidman[3].* 1) Department of Pediatrics, Div Human Genetics, , CA; 2) Department of Developmental and Cell Biology Univ California, Irvine, Irvine; 3) Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts.

ABSTRACT Holt-Oram syndrome (HOS; OMIM#142900) is an autosomal-dominant condition characterized by cardiac malformations and radial ray skeletal abnormalities. Cardiac defects range from atrial or ventricular septal defects to complex cardiac defects; skeletal anomalies include thumb abnormalities such as triphalangeal thumbs to severe radial or ulnar defects. Mutations in TBX5 cause this condition. TBX5 is a member of the T-box transcription factor family. To understand pathogenesis of TBX5 mutations in HOS, we sequenced the coding regions and the intron/exon boundaries in familial and sporadic cases with HOS. We identified four novel mutations including one composite heterozygous mutation. This represents the first case of HOS with composite heterozygous mutation in live birth. Furthermore, we found no mutations in TBX5 in two families with evidence for linkage to 12q. These studies demonstrate that composite heterozygous mutation is viable in HOS and that mutation may harbor outside of the TBX5 coding region. Further analysis is needed to study the pathogenesis of composite heterozygous mutation and to search for mutation in other regions of the TBX5 gene.

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R849W-TIE2 mutation is frequently observed in cutaneomucosal venous malformation (VMCM). *V. Wouters[1], A. Irrthum[1], L.M. Boon[1, 2], J. Murphy[3], P. Rieu[4], L. Kangesu[5], A. Pennington[6], J.B. Mulliken[7], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology and University of Louvain Medical School, Brussels, Belgium; 2) Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires St-Luc, University of Louvain Medical School, Brussels, Belgium; 3) Hospital for Sick Children, Toronto, Canada; 4) Kinderchirugie, UMC, St-Rabdoud, University of Nijmegen, Holland; 5) The St-Andrews Centre for Plastic Surgery and Burns, Essex Hospital, UK; 6) Department of Surgery, St-Vincents Hospital, University of Melbourne, Australia; 7) Division of Plastic Surgery, Childrens Hospital and Harvard Medical School, Boston, MA, USA.

Venous malformations (VM) are low-flow vascular lesions consisting of disorganized thin-walled vascular channels. They usually occur sporadically, but sometimes as an autosomal dominant condition termed cutaneomucosal venous malformation (VMCM; OMIM 600195). TIE2 gene, localized in 9p21, encodes an endothelial cell-specific receptor tyrosine kinase. TIE2 is required for blood vessel formation and maintenance. Two activating mutations, R849W and Y897S, in the kinase domain of TIE2 co-segregate with VMCM in four families (Vikkula et al., 1996; Calvert et al., 1999). We have identified a TIE2 mutation in four novel families with VMCM. In each of them, the identified mutation was the same R849W substitution previously reported (Vikkula et al., 1996). Thus, altogether 8 families with VMCM present a TIE2 mutation, 7 of which the R849W substitution. In 3 out of 5 of the VMCM families tested with polymorphic markers, we identified haplotype sharing in a 1.4 Mb region, suggesting that the R849W mutation in these families is an ancestral mutation. However, in the other two families the haplotype was different. Thus, the R849W mutation may represent one of few changes in TIE2 that can give rise to a signalling defect that results in VMCM, or the mutation site is a hot spot for mutations in TIE2. It remains to be established if TIE2 has any role in sporadic VM, via e.g. somatic mutations (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.ac.be/vikkula).

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The Myosin Binding protein C R943X and 2864delCT founder mutations may confer relatively severe hypertrophic cardiomyopathy. *Y.M. Hoedemaekers[1,2], E. Biagini[2], W. Deelen[1], H.J. Meijers-Heijboer[1], M.J.M. Kofflard[2], D.J.J. Halley[1], F.J. ten Cate[2], D. Dooijes[1].* 1) Dept. of Clinical Genetics, Erasmus MC, the Netherlands; 2) Thoraxcenter, Erasmus MC, Rotterdam, the Netherlands.

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant monogenic disease, with incomplete penetrance and a variable phenotypic expression. It is the most common cause of sudden death (SD) in the young. Mutations in more than 11 genes, mostly encoding sarcomere proteins, have been shown to cause HCM. Many of these mutations arise independently and founder effects are rarely observed. Earlier studies associated mutations in the sarcomere Myosin Binding Protein C gene (MYBPC3) with late onset HCM and a relatively benign clinical expression. We performed sequence analysis of the MYBPC3 gene in 189 unrelated HCM index patients. A pathogenic mutation was identified in 52% of the patients. In addition to the previously identified 2373insG founder mutation, which was found in 17% of the patients, we identified the R943X and 2864delCT mutations in 6% and 7% of the analysed HCM population respectively. Subsequent extended haplotype analysis demonstrated these mutations to be additional HCM founder mutations in the Dutch population. Relevant clinical parameters were analysed in 19 carriers of the R943X mutation and 16 carriers of 2864delCT. HCM associated SD was observed in 50% of the families analysed. Mean age at diagnosis was 3912 years for the R943X mutation and 4515 years for the 2864delCT mutation. Validation was NYHA class II or higher at presentation in 42% and 63% of the respective mutation carriers. Conclusions: In addition to the 2373insG mutation, the R943X and 2864delCT mutations are MYBPC3 founder mutations, together accounting for one third of all HCM patients in the Netherlands. Variability of age at onset and clinical symptoms were observed in the R943X and 2864delCT mutation carriers and a surprisingly high percentage of sudden death was seen in the affected families. In contrast to earlier studies reporting MYBPC3 mutations to be relatively benign, both HCM mutations are associated with the development of heart failure symptoms and possible malignant course of the disease.

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Mutation study on BMPR2 and ALK1 genes in Japanese patients with primary pulmonary hypertension. *H. Morisaki[1], N. Nakanishi[2], S. Kyotani[2], H. Tomoike[2], T. Morisaki[1,3].* 1) Dept Bioscience, NCVC Research Inst, Suita, Osaka, Japan; 2) Dept Cardiovascular Medicine, NCVC, Suita, Osaka, Japan; 3) Dept Molecular Pathophysiology, Osaka U Graduate Sch Pharmaceutical Sciences, Suita, Osaka, Japan.

Primary pulmonary hypertension (PPH) is a potentially lethal disorder, in which heterozygous mutations within the bone morphogenetic protein type II receptor (BMPR-II) gene (BMPR2) have been identified. We performed the molecular study of ALK1 gene as well as BMPR2 gene in 59 Japanese patients with PPH, including 7 familial PPH cases. We also performed the molecular study in 61 cases with secondary pulmonary hypertension (SPH). We identified mutations of BMPR2 gene in 23 cases'i39%'j of patients with PPH, while only one patient was found to have BMPR2 mutation. Regarding ALK1 gene, three cases (5%) of patients with PPH was found to have mutations, while only one patient with SPH was found to have a mutation. Those mutations are predicted to result in a premature termination codon or substitution of an amino acid residue. Furthermore, we identified functional relevance of those mutations by using cellular model for TGFb/BMP signaling. Based on these results, there is considerable genetic heterogeneity of PPH, and further functional study will be needed to identify pathophysiological mechanism of pulmonary hypertension.

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BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *M.A. Aldred[1], J. Vijayakrishnan[1], V. James[1], M.A. Gomez-Sanchez[2], G. Martensson[3], N.W. Morrell[4], R.C. Trembath[1].* 1) Div. of Medical Genetics, University of Leicester, UK; 2) Hospital Universitario 12 de Octubre, Madrid, Spain; 3) Sahlgrenska University Hospital, Gothenburg, Sweden; 4) Div. of Respiratory Medicine, University of Cambridge School of Medicine, Cambridge, UK.

Mutations of the *BMPR2* gene predispose to pulmonary arterial hypertension (PAH), a serious, progressive disease of the pulmonary vascular system. However, despite the fact that most PAH families are consistent with linkage to the *BMPR2* locus, sequencing only identifies mutations in some 55% of familial cases and approximately 26% of cases without a family history (idiopathic or IPAH). We therefore conducted a systematic analysis for larger gene rearrangements in panels of both familial and idiopathic PAH cases that were negative on sequencing of coding regions. Analysis of exon dosage across the entire gene using multiplex ligation-dependent probe amplification (MLPA)identified six novel deletions and enabled full characterization at the exon level of three previously reported deletions. Overall, *BMPR2* deletions were identified in 6 of 21 mutation-negative families (28.6%) and 4 of 87 IPAH cases (4.6%), suggesting that gross rearrangements underlie around 12% of all FPAH cases and 3% of IPAH. Deletions occurred at multiple different sites throughout the gene and while breakpoints for five deletions fell within intron-1, further analysis showed them to be distinct. Thus there was no evidence for breakpoint clustering. Notably, one deletion encompassed all functional protein domains and is predicted to result to in a null mutation, providing the strongest support yet that the predominant molecular mechanism for disease predisposition is haploinsufficiency. Dosage analysis of *BMPR2* should now be considered an integral of part of the molecular work-up of PAH patients, particularly those with a previous family history.

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IDENTIFICATION OF NOVEL PKP2 MUTATIONS IN DUTCH ARVC PATIENTS. *M.R. Nelen[1], M. Entius[1], J.J. van der Smagt[1], R.N.A. Hauer[2], J.K. Ploos van Amstel[1], P.A. Doevendans[2], R.J. Sinke[1].* 1) Dept. of Medical Genetics,UMC Utrecht, Utrecht, The Netherlands; 2) Dept. of Cardiology,UMC Utrecht, Utrecht, The Netherlands.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a complex disorder and is associated with sudden unexpected death in young individuals. Patients with ARVC present with a gradual loss of myocytes that are replaced by fatty and fibrous tissue. In about 50% of ARVC-cases affected family members can be identified, usually consistent with an autosomal dominant mode of inheritance with reduced penetrance. The exact incidence of ARVC is unknown. A variety of chromosomal locations have been identified leading to the recognition of eight ARVC subtypes. Recently, the plakophilin 2 (PKP2) gene, an essential armadillo-repeat protein of the cardiac desmosome, was shown to be mutated in 27% of patients with ARVC (Gerull et al. 2004). Our goal was to assess the prevalence of PKP2 mutations in 22 Dutch patients that fulfilled the criteria for ARVC. The entire coding sequence and exon flanking sequences of the plakophilin 2 gene were screened for mutations using direct sequencing. In 6 unrelated probands 6 different disease releated mutations were identified (27%). These included 2 missense, 3 nonsense, and 1 frameshift mutation. One proband revealed 2 mutations: in addition to a causative intron 12 splice-site mutation (IVS12+1G>A) we also identified an amino acid substitution (Gln62Lys). Five of the mutations have not yet been reported. In conclusion, PKP2 mutations can be identified in up to 30% of Dutch patients fulfilling the ARVC criteria. These results will facilitate early recognition of persons at risk and future studies of potential genotype-phenotype relationships.

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A 12-gene DNA resequencing chip for molecular diagnosis of hypertrophic cardiomyopathy. *J.L. Blouin[1], R. Lyle[2], U. Sigwart[3], A. Munoz[2], C. Gehrig[2], R. Lerch[2], M. Beghetti[4], J. Sztajzel[3], S.E. Antonarakis[1,2], S. Fokstuen[1].* 1) Medical Genetics, Univ. Hospitals of Geneva, Switzerland; 2) Genetic Medicine and Development, University of Geneva-School of Medicine, Switzerland; 3) Cardiology, Univ. Hospitals of Geneva, Switzerland; 4) Pediatric Cardiology, Univ Hospitals of Geneva, Switzerland.

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder (prevalence 1/500) with a wide range of clinical features from a life-long asymptomatic course to chronic heart failure or sudden cardiac death. To date mutations associated with HCM have been identified in 14 genes, the majority of which (88%) are single nucleotide substitutions within exons, splice sites, or promoter regions. In clinical practice, molecular diagnosis of HCM is of considerable benefit for diagnosis, prognosis or predictive testing. However, the genetic heterogeneity of HCM means that mutation detection by classical methods is time-consuming and difficult to realise in a routine diagnostic molecular laboratory. The recent development of DNA resequencing microarrays allows the simultaneous analysis of multiple genes in a single experiment. We have developed such an array for fast (few days) and high-throughput molecular diagnosis of HCM. Our array comprises all coding exons (160), splice-site, and promoter regions of 12 genes mutated in HCM. We report the results of a first series of 25 unrelated patients with HCM. Mean Left-Ventricular-Wall-Thickness (LVWT) was 20 mm (14-29). We successfully assigned base calls at 95% of 26000 base positions. So far we confirmed at least 15 functional variants. One amino acid change (T1377M) in exon 30 of MYH7 is a known mutation. All the other variants are novel (12 amino acid changes in the genes MYBPC3, MYH7, MYL3, and TNNT2, 1 splice site variant -IVS25+1G>T- in MYBPC3, and 1 variant in the promoter region of PLN). Furthermore we detected at least 15 known silent SNPs. These first findings prove the utility and the performance of the HCM array as a high-throughput tool for the detection of nucleotide substitutions in clinical practice. The evaluation of further variants as well as of further samples is in progress.

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Large phenotypic variability in 24 novel families with CM-AVM caused by a mutation in RASA1 gene. *N. Revencu[1,2], L. Boon[1,3], O. Enjolras[4], J.B. Mulliken[5], M. Vikkula[1], and the CM-AVM study group.* 1) Lab of Human Molecular Genetics, Christian de Duve Inst of Cellular Pathology, Université catholique de Louvain, Brussels, Belgium; 2) Centre for Human Genetics, Clin Univ St. Luc, Brussels, Belgium; 3) Centre for Vascular Anomalies, Clin Univ St. Luc, Brussels, Belgium; 4) Consultation des Angiomes, Lariboisière Hospital, Paris, France; 5) Vascular Anomalies Center, Div of Plastic Surgery, Childrens Hospital, Harvard Medical School, Boston, Ma 02115, USA.

Capillary Malformation-Arteriovenous Malformation (CM-AVM) (MIM 608354) is a recently identified phenotype characterised by atypical capillary malformations (CM) associated with either arteriovenous malformation, arteriovenous fistula, or Parkes Weber syndrome (PWS) (Eerola et al., 2003). The most prominent feature is the presence of atypical CMs, which are small, round-to-oval in shape and pinkish-red in color. We showed that the CM-AVM phenotype is caused by mutations in the RASA1 gene and reported 6 different mutations. We report here 23 distinct mutations in 24 novel families with CM-AVM. The mutations were spread throughout the gene and 22 of them caused a premature stop codon: 9 nonsense, 7 frameshift and 6 splicing mutations, suggesting a loss of function as the most likely mechanism. In one family, a missense mutation was identified (Y426C). Interestingly, 8 mutations were clustered in 2 exons, part of the RasGap domain: 3 in the exon 17 and 5 in the exon 19. Fifty-two individuals carrying a RASA1 mutation were identified in these 24 families: 37 had CMs and 15 had, in addition, a high-flow lesion (5 AVM, 1 AVF and 9 PWS). In conclusion, 28 different RASA1 mutations have been identified so far, clearly delineating the CM-AVM phenotype. A considerable proportion of patients (27%) had, besides the atypical CMs, a high-flow lesion. Its specific clinical, radiological and genetic characteristics should avoid the confusion with other vascular anomalies, such as hereditary benign telangiectasia or Klippel-Trenaunay syndrome. Our data also suggests that the CM-AVM phenotype could be a relatively frequent genetic disorder. (vikkula@bchm.ucl.ac.be).

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Irish wolfhound Cardiomyopathy with Atrial Fibrillation maps to CFA23. *P.M. Jakobs[1], M. Bestwick[1], S. Nelson[1], M. Winther[1], E. Ostrander[2], R.E. Hershberger[1], M. Litt[3].* 1) Medicine, Cardiology, Oregon Health & Science Univ, Portland, OR; 2) NHGRI, NIH, Bethesda, MD; 3) Molecular & Medical Genetics, Oregon Health & Science Univ, Portland, OR.

In collaboration with dog owners and breeders we obtained clinical data and blood for DNA from a large Irish Wolfhound (IW) pedigree in which atrial fibrillation (AF) with dilated cardiomyopathy (DCM) is inherited. The disease, occurring naturally in IWs, offers a valuable opportunity to study a large animal model that closely resembles human genetic cardiomyopathy. Our working pedigree includes 142 IWs of which 45 dogs have AF and/or DCM. Overlapping 3-point linkage analysis using linkmap and assuming 1Mb = 1cM, was applied to data from 452 microsatellite markers. Assuming autosomal dominant inheritance with affecteds only, we excluded approximately 75% of the genome. A region with positive lodscores was identified on CFA23. Multipoint linkage analysis using an affected only model revealed a region with a multipoint lodscore exceeding 3. The critical region is between markers AHTK253 and 23_12.71 with a maximum multipoint LOD score of 3.44 at FH3671. We have yet to identify the causative gene in this 1.43 Mb chromosomal region, and our first approach will focus on the sequencing of SCN5A, a positional candidate gene, mutations in which have been shown to cause human dilated cardiomyopathy and conduction system disease.

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Interaction between *Pkd1* and *Fbn1* in the pathogenesis of aortic aneurysm. *J. Moslehi[1], C. Wang[1], D. Judge[1], K. Piontek[1], J. Deng[1], G. Germino[1], T. Watnick[1], H.C. Dietz[1,2].* 1) Johns Hopkins Univ, Baltimore, MD; 2) HHMI, Bethesda, MD.

Marfan syndrome (MFS) is a systemic disorder of connective tissue caused by mutations in the gene encoding fibrillin-1 (*FBN1*), with cardinal manifestations including proximal aortic aneurysm and dissection. Autosomal dominant polycystic kidney disease (ADPKD) is also associated with vascular disease including aortic and intracranial aneurysm. The majority of cases are caused by mutations in the gene encoding polycystin-1 (*PKD1*). The precise function of polycystins in the vasculature has not been defined. We bred mice harboring mutant alleles of *Pkd1* and *Fbn1*, and the offspring were sacrificed at 6 months of age. *Pkd1*+/- mice had a mild increase in aortic wall thickness compared to controls (41.5 M vs 27.6 M for WT). *Fbn1*+/- mice had greater aortic wall thickness (50.3 M) and aneurysm. Compound heterozygous mice had even greater aortic wall thickness (60.8 M) and aneurysm compared to either isolated het. In addition, the compound het mice had prominent inflammation at the site of aortic aneurysm not seen in either isolated het. Based on our prior recognition of increased TGF activity in fibrillin-1 deficient mice, we speculated that an interaction between *Pkd1* and *Fbn1* might manifest further up-regulation of this pathway by *Pkd1* mutation. We found that aortic sections from these mice demonstrated increased active TGF and increased TGF signaling, as evidenced by nuclear enrichment of pSmad2/3 and increased expression of PAI-1 and CTGF when compared with either single het. In order to test whether polycystin-1 was capable of regulating TGF activity, we induced recombinant expression of either wild-type or C-truncated (P89) polycystin-1 in NIH3T3 cells, and stimulated with TGF1 (4ng/ml). In order to quantify activation of TGF signaling, western blots were probed with antibodies to Smad4 and pSmad2/3. We found that *PKD1*-transfected cells had a significantly blunted response to TGF stimulation when compared with either vector or P89-transfected cells, despite similar levels of expression. Our results suggest that polycystin-1 modulates activity of the TGF signaling pathway, and may thus contribute to the pathogenesis of aortic aneurysm.

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Locus heterogeneity in ASD associated with AV-block. *L. De Roy[2], I. Gutierrez-Roelens[1], C. Ovaert[2], Th. Sluysmans[2], K. Devriendt[3], H.G Brunner[4], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular and Pathology, Brussels, Belgium; 2) Division of Pediatric Cardiology and Cardiology, Cliniques universitaires St Luc, University of Louvain Medical School, Brussels, Belgium; 3) Center for Human Genetics, University of Leuven, Leuven, Belgium; 4) Department of Human Genetics, University Medical Center St Radboud, Nijmegen, The Netherlands.

The prevalence of congenital heart defects is approximately 1% of all live births. Identifying the genes responsible for cardiac malformation is the first step to understand pathogenesis. Heterozygous mutations in the CSX/NKX2-5 gene have been identified to cause atrial septal defect (ASD) and/or atrioventricular conduction disturbance in some families. There is great variability in expressivity between the patients with a CSX/NKX2-5 mutation. We screened 4 sporadic patients and 5 index cases of families with ASD and/or conduction defects. In one of them, a CSX/NKX2-5 mutation was identified. This novel mutation was inherited in a 3-generation family causing 5 individuals to present cardiac symptoms ranging from ASD to arrhythmias. In the 4 other families affected with left ventricular non-compaction cardiomyopathy, hypoplastic left heart syndrome and ASD with AV-block, no CSX/NKX2-5 mutation was found. Two of the 4 families presented a phenotype characteristic of CSX/NKX2-5 mutated individuals. Deletion of the whole gene was also excluded. These two families have also been screened for mutations in CRELD1, FOG2 and BMP4, candidate genes for septation. As no mutation was identified in these genes either, the data strongly suggests genetic heterogeneity in ASD with conduction defects. (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.ac.be/vikkula/).

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No evidence for mutations in five candidate genes, GATA4, FOG2, CRELD1, HEY2 and BMP4 as a common cause of structural cardiac defects. *Th. Sluysmans[2], C. Ovaert[2], I. Gutierrez-Roelens[1], K. Devriendt[3], H.G Brunner[4], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology; 2) Division of Pediatric Cardiology, Cliniques universitaires St Luc, University of Louvain Medical School, Brussels, Belgium; 3) Center for Human Genetics, University of Leuven, Leuven, Belgium; 4) University Medical Center St Radboud, Department of Human Genetics, Nijmegen, The Netherlands.

Cardiac septation involves the closure of direct communications between left and right atria, ventricles and subarterial channels, and the development of the atrioventricular junction and the ventriculoarterial junction. Disruption of these processes gives rise to cardiac septal defects. Several transcription factors including CSX/NKX2-5, TBX5, TBX1, JAG1, SALL4, EVC1 and EVC2 regulate septal formation. Yet, in non-syndromic septal defects it has been very difficult to identify predisposing genetic factors, and in man clear mutations have so far only been identified in the CSX/NKX2-5 gene, usually in association with conduction defects. Recently, three other genes (GATA4, FOG2, CRELD1), on the basis of certain non-synonymous amino-acid changes, were suggested to be responsible of isolated septal defects in man. In mouse, inactivation or modification of two other genes results in septal defects (HEY2, BMP4). In this study, we screened these five genes (GATA4, FOG2, CRELD1, HEY2 and BMP4) in a series of 66 patients affected with a structural, congenital cardiac malformation, mostly a septal defect. Twenty-nine nucleotide changes were identified, resulting in 12 synonymous and 10 non-synonymous amino acid changes. None of these nucleotide variations was considered a mutation, as they were either identified in dbSNP, dbEST, or the amino acid change did not occur in a conserved region. For some of them we cannot exclude an effect on RNA stability or abnormal splicing. We conclude that none of these five genes is a major cause of structural cardiac defects in man. (vikkula@bchm.ucl.ac.be) (http://www.icp.ucl.ac.be/vikkula).

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Novel glycogen synthase kinase 3 (GSK3) and ubiquitination pathways in progressive myoclonus epilepsy. *H.T. Lohi[1], L. Ianzano[1], X.C. Zhao[1], E.M. Chan[1], J. Turnbull[1], S.W. Scherer[1], C.A. Ackerley[3], B.A. Minassian[1,2].* 1) Genetic and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Paediatrics (Neurology), The Hospital for Sick Children,Toronto,ON, Canada; 3) Department of Pathology and Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada.

Lafora progressive myoclonus epilepsy, caused by defective laforin or malin, presents insidiously in normal teenagers with cognitive decline, followed by rapidly intractable epilepsy, dementia and death. Pathology reveals neurodegeneration with neurofibrillary tangle formation and Lafora bodies (LB). LB are deposits of starch-like polyglucosans, insufficiently branched and hence insoluble glycogen molecules resulting from glycogen synthase (GS) overactivity relative to glycogen branching enzyme activity. We previously made the unexpected observation that laforin, in the absence of which polyglucosans accumulate, specifically binds polyglucosans. This suggested that laforins role is to detect polyglucosan appearances during glycogen synthesis and initiate mechanisms to downregulate GS. Glycogen synthase kinase 3 (GSK3) is the principal inhibitor of GS. Dephosphorylation of GSK3 at Ser 9 activates GSK3 to inhibit GS through phosphorylation at multiple sites. Glucose 6-phosphate is a potent allosteric activator of GS. At times of glucose plenty, its levels are high, and its activation of GS overrides any phospho-inhibition. Here we show that laforin is a GSK3 Ser 9 phosphatase, and therefore capable of inactivating GS through GSK3. We also show that laforin interacts with malin, and that malin is an E3 ubiquitin ligase that binds GS. We propose that laforin, in response to appearance of polyglucosans, directs two negative feedback pathways, one, polyglucosan-laforin-GSK3-GS, to inhibit GS activity, and the other, polyglucosan-laforin-malin-GS to remove GS through proteasomal degradation.

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Genetic and evolutionary analysis of the *ABCC6* gene and pseudoxanthoma elasticum (PXE). *H. Lou[1], J. Bergeron[2], L. Thomas[1], S.F. Terry[3], P.F. Terry[3], M. Dean[1].* 1) Lab Genomic Diversity, NCI-FCRDC, Frederick, MD; 2) SAIC-Frederick, Frederick, MD; 3) PXE International, Washington, DC.

The *ABCC6* gene is a member of the ATP-binding cassette (ABC) transporter superfamily and encoding multidrug resistance protein 6 (MRP6). Although the function of ABCC6 is unknown, mutations in the human *ABCC6* gene result in a heritable disorder principally affecting the skin and eyes, pseudoxanthoma elasticum (PXE). To explore the inheritance of PXE and the penetrance of *ABCC6* mutations, we typed several polymorphisms in and flanking the *ABCC6* gene in 16 PXE pedigrees. We identified two potential non-penetrant cases that one male and one female that require followup. An overrepresentation of affected females was found both in the probands and in the siblings of probands. This was not quite statistically significant in this sample.
To develop an animal model for PXE, the full-length zebrafish *Abcc6* cDNA was successfully cloned and sequenced. We quantitated the mRNA expression from a variety of zebrafish tissues including skin, head, eyes, bone, organs, eggs, muscle, and embryos using real-time RT-PCR. The highest levels of *Abcc6* mRNA were found in the skin, followed by head, eyes and embryo. Furthermore, we measured *Abcc6* mRNA in embryos 6 days post-fertilization (dpf). The level of *Abcc6* mRNA in embryos was shown to be intermediate between that of the eyes and bone. In situ hybridization revealed expression of the Abcc6 in discrete areas of the skin, consistent with the RT-PCR data. Our results demonstrate the presence of Abcc6 in a variety of tissues, including those tissues affected in PXE patients, suggesting a possible role for ABCC6 in the normal assembly of extracellular matrix components. The sequencing data contribute to the development of an animal model for PXE and identification of the function of ABCC6.

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Detection of a heterozygous SALL1 deletion proves the contribution of a SALL1 dosage effect in the pathogenesis of Townes-Brocks syndrome. *W. Borozdin[1], K. Steinmann[1], B. Albrecht[2], J. Kohlhase[1].* 1) Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany; 2) Institute for Human Genetics, Duisburg-Essen University, Essen, Germany.

Townes-Brocks syndrome (TBS, OMIM #107480) is an autosomal dominantly inherited malformation syndrome characterized by anal, renal, limb, and ear anomalies caused by mutations in the putative zinc finger transcription factor gene SALL1. The mapping of the TBS gene locus to chromosome 16q12.1 was achieved by identification of two cytogenetic abnormalities, both involving a breakpoint at 16q12.1. Based on the sole observation of truncating mutations in the SALL1 gene in TBS patients and the association of TBS with chromosomal anomalies, haploinsufficiency was assumed as the pathogenic mechanism leading to TBS. However, Sall1 knock-out mice did not show any phenotype in the heterozygous situation and solely severe kidney defects in the homozygous mutants. Instead, another mouse model mimicking the human mutations revealed a TBS-like phenotype in the heterozygous mutants. Furthermore, biochemical evidence suggested that truncated SALL1 proteins do exist and possibly lead to the phenotype by a dominant-negative action as well as by interfering with other SALL proteins. This hypothesis could not integrate the observation of cytogenetic anomalies in TBS patients. Here we show the first patient with a heterozygous deletion of the SALL1 gene. The deletion was not visible on cytogenetic analysis and involves all coding exons of SALL1 as well as several kb of downstream sequence, as detected by quantitative real time PCR. The patient presented with a mild TBS-like phenotype, consisting of a small ear, bilateral triphalangeal thumbs and an anteriorly positioned anus. While further patients with deletions are needed to clarify if the severe TBS phenotype could also be associated with a SALL1 deletion, our data prove that a SALL1 dosage contributes significantly to human TBS and indicate that truncated proteins might lead to a more severe TBS phenotype by interacting with other SALL proteins.

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No evidence for association between the serotonin transporter promoter polymorphism and pulmonary arterial hypertension. *R.D. Machado[1], E. Gruenig[2], B. Janssen[3], R. Koehler[3], C.G. Elliott[4], M. Humbert[5], F. Soubrier[6], N.W. Morrell[7], R.C. Trembath[1].* 1) Division of Medical Genetics, University of Leicester, Leicester, Leicestershire, United Kingdom; 2) Department of Cardiology and Pneumology, University Hospital Heidelberg, Germany; 3) Institute of Human Genetics, University of Heidelberg, Germany; 4) LDS Hospital and the University of Utah, Salt Lake City, USA; 5) Service de Pneumologie, UPRES EA2705, Hopital Antoine Beclere, France; 6) Laboratoire dOncogénétique et dAngiogénétique Moléculaire, GH Pitié-Salpêtrière, 47 bd de lhôpital, France; 7) Respiratory Medicine Unit, University of Cambridge, UK.

Pulmonary arterial hypertension (PAH) is a severe disorder characterised by a sustained elevation of pulmonary arterial resistance leading to cardiac failure. PAH may segregate within families (FPAH), develop spontaneously (IPAH) or be observed in the setting of associated complications (APAH). Mutation of the BMPR2 gene, encoding a type II TGF-beta receptor, has been established as the major genetic factor underlying the condition. However, mutant alleles exhibit reduced age- and sex-dependent penetrance indicating the requirement for additional environmental and/or genetic modifiers in disease pathogenesis. Recently, a putative association between the mitogen serotonin, and, specifically, its transporter (5-HTT) with the pathogenesis of PAH has generated the important hypothesis that 5-HTT might act as a genetic modifier of disease. To comprehensively investigate the presence of a genetic association we compared the distribution of a functional INDEL polymorphism in the promoter of the 5-HTT gene between FPAH (n=145), IPAH (n=282) and APAH (n=136) cohorts, to ethnically matched controls (n=353). The analysis was extended to examine the possible contribution of 5HTT variation to age of onset of clinical disease. We obtained no evidence for association either with the three clinical classes of PAH (P>0.5)or with age of disease onset. The absence of association in these large case groups provides compelling evidence against a role for the 5HTT gene in PAH susceptibility or as a significant modifier of disease progression.

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The Role of the GNRH1 gene in patients with Idiopathic Hypogonadotropic Hypogonadism. *B.S. Villarreal, L. Chorich, L. Layman.* Section of Reproductive Endocrinology, Infertility, & Genetics, Dept. OB/GYN, Developmental Neurobiology Program, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA.

Idiopathic hypogonadotropic hypogonadism (IHH) is a rare disorder characterized by an irreversible delay of puberty (age 17 for females and 18 for males) and accompanying low levels of the serum gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). Other pituitary hormone levels are typically normal, and pituitary tumors are absent. Because of its role in regulating gonadotropins, the GNRH1 (gonadotropin releasing hormone-1) gene represents the ideal candidate gene for mutations that may cause IHH. GNRH1 encodes for a signal peptide, the decapeptide GnRH, and GAP (GnRH-associated peptide), both of which increase gonadotropin secretion. Although there have been some studies of IHH families for mutations in the GNRH1 gene, to date, all of these were performed upon a relatively small sample size and failed to reveal any mutations in IHH patients. Because of the important orchestrating role of GnRH in the synthesis and secretion of gonadotropins and the identification of a Gnrh1 deletion in the hypogonadal mouse, we proposed to study a large sample of unrelated IHH probands for mutations. Total RNA extracted from lymphoblastoid cells from 80 unrelated IHH probands was subjected to reverse transcriptase PCR (RT-PCR) using a single primer set. The amplified region contained the entire coding region for GnRH and GAP, as well as part of the signal peptide. PCR products were electrophoresed on agarose gels to confirm the presence of an appropriately sized band, and sequenced. No sequence variation was present in any patient, including the previously identified polymorphism in the signal peptide at codon 16. We conclude that mutations in the coding region of the GNRH1 gene are extremely uncommon in IHH patients, as are mutations that affect splicing. These findings corroborate the observation that no human hypothalamic releasing factor to date possesses mutations in human genetic disease.

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Characterizing the effect of *Tcof1* on cell proliferation using a neuroblastoma cell line. *L. Li, R. Shiang.* Human Genetics, Virgina Commonwealth University, Richmond, VA.

Treacher collins syndrome (TCS) is an autosomal dominant craniofacial development disorder. Previously, we identified candidate *Tcof1* downstream genes using microarray analysis by manipulating *Tcof1* level in a murine neuroblastoma (NB) cell line. Candidate downstream genes include cell cycle genes as well as transcription factors *Cnbp* and *Tbx2*, which are known to affect cell cycle through c-myc and p19-Mdm2/p21 pathway respectively. To further characterize the effects of *Tcof1* level changes on NB cell proliferation, growth curves were generated by counting cell numbers, BrdU incorporation was used to determine rate of DNA replication and analyzed with flow cytometry, and TUNEL assay was used to determine apoptosis levels. Western blot analysis was used to detect protein level changes of candidate downstream pathway genes. Both overexpress and knockdown of *Tcof1* caused decreased proliferation of NB cells. Overexpression of Tcof1 caused an increased apoptosis level as well as DNA replication level, which indicate the cells are likely entering apoptosis from the G2 stage of the cell cycle. Inconsistent with mouse model results, knockdown of Tcof1 caused a slightly decreased apoptosis level and no apparent difference in DNA replication level compared to wild type. Heterozygous *Tcof1* knock out mice showed an increased apoptosis level at highest *Tcof1* expression sites. This may be caused by the difference between neural crest cells *in vivo* and the NB cell line we used. Western blot analysis showed c-myc level changed coordinately with Tcof1. There are no apparent changes on p21 (Cdkn1a) and Mdm2 levels. Our results suggested an optimal Tcof1 level is required for cell proliferation and survival. Results indicated that *Tcof1* may affect cell proliferation through the c-myc pathway. We are investigating if *Cnbp* and *Tbx2* are involved in the effects of *Tcof1* on cell proliferation and survival.

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Molecular studies of candidate genes involved in defects of human spermatogenesis. *W. Lissens[1], K. Stouffs[1], A. Willems[1], H. Tournaye[2], A. Van Steirteghem[2], I. Liebaers[1].* 1) Center for Medical Genetics; 2) Center for Reproductive Medicine, Univ Hosp of the Vrije Universiteit Brussel, Brussels, Belgium.

In an attempt to identify genetic causes of human male infertility due to severely defective spermatogenesis, we have studied several candidate genes. We focused on genes that are only expressed in testicular tissue and that are located on the X chromosome. Infertile patients for these studies were selected based on the expression pattern of the corresponding genes in mice and their likely consequence on spermatogenesis in case of deficiency. Genes studied were TEX13A, TEX13B and TAF7L. Testis-specific expression of genes was studied by PCR using gene-specific primers and commercially available or homemade cDNAs prepared from human testicular tissue. If appropriate, intronic primers were designed for PCR amplification and for sequencing of the genes. PCR amplification of cDNA failed to show expression of the human TEX13A and TEX13B genes. Published data on these genes possibly refer to DNA contamination of the cDNA preparations because primers were designed in intron-less regions. No further studies of these genes were performed. In contrast, TAF7L cDNA was specifically found in testicular tissue. Mutation analysis of the gene was performed in 27 patients with a maturation arrest of spermatogenesis. Four changes in comparison to the normal sequence were found, and three of them proved to be polymorphisms by studying 60-120 normal fertile males as controls. One change, c.1373G>A predicting Arg458Cys, was found in 2 patients but not in 103 male controls. From mouse studies, it is known that TAF proteins have an important function in the regulation of transcription. TAF7L is expressed from the stage of spermatogonia until the stage of round spermatids, and during meiosis, a shift of the protein from the cytoplasm to the nucleus is observed. If the expression of the gene is similar to that in mouse, defects in the gene are likely to cause maturation arrest at the level of meiosis or early spermatid differentiation. It will be important to prove the role of the TAF7L protein in the human, and to verify the possible effect of the Arg458Cys change on its function.

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The role of Biogenesis of Lysosome-Related Organelles Complexes (BLOCs) in human genetic disease. *A. Helip Wooley[1], H. Dorward[1], W. Westbroek[1], R. Boissy[2], M. Huizing[1], W.A. Gahl[1].* 1) NHGRI, NIH, Bethesda, MD; 2) Univ. of Cin., OH.

Hermansky-Pudlak syndrome (HPS) is a disorder of impaired lysosome-related organelle (LRO) biogenesis. Defects in melanosomes in melanocytes and dense bodies in platelets are hallmarks of the disease and manifest clinically with oculocutaneous albinism and a bleeding diathesis. There are seven subtypes of human HPS described, with great variability in severity, irrespective of mutation. HPS-3, HPS-5 and HPS-6 generally have mild hypopigmentation with little or no bleeding, while HPS-1 and HPS-4 have severe albinism, bleeding and early death from pulmonary fibrosis. The correlation between disease subtype and severity is becoming more apparent as we learn how HPS proteins interact and function in Biogenesis of Lysosome-Related Organelles Complexes or BLOCs. HPS3, HPS5 and HPS6 proteins are found in BLOC-2. EM of melanocytes from patients with these subtypes reveals fewer mature melanosomes and an abundance of small tyrosinase (tyr)-positive vesicles mislocalized throughout the cell. We have found that HPS3 has a functional clathrin-binding domain and that HPS3 co-localizes with clathrin on small vesicles. We hypothesize that BLOC-2 binds the small, tyr-positive vesicles and escorts them to early stage melanosomes for delivery of their cargo. HPS1 and HPS4 are members of BLOC-3. The small mis-localized vesicles seen in BLOC-2 deficient cells are absent from BLOC-3 deficient melanocytes, but large, tyr-containing multilamellar compartments are present. Immunofluorescence studies reveal that some melanosomal and lysosomal proteins remain in the trans-Golgi region. It appears that BLOC-3 deficient cells fail to form the small vesicles that serve as transport intermediates for melanosomal proteins. In contrast, BLOC-2 deficient cells form these vesicles, but they do not properly traffic to and/or fuse with maturing melanosomes. Thus, BLOC-3 acts at an earlier stage of LRO biogenesis, so HPS-1 and HPS-4 result in clinically more severe disease compared with BLOC-2 defects (HPS-3, -5 and -6). HPS illustrates a new paradigm of using cell biology to understand human genetic disease.

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Altered membrane trafficking in genetically accurate mouse and cerebellar cell culture models of juvenile neuronal ceroid lipofuscinosis. *S.L. Cotman[1], Y. Cao[1], E. Fossale[1], P. Wolf[1], J.A. Espinola[1], J.M. Weimer[2], D.A. Pearce[2], M.E. MacDonald[1].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Charlestown, MA; 2) Center for Aging and Developmental Biology, University of Rochester Medical Center, Rochester, NY.

Juvenile neuronal ceroid lipofuscinosis (JNCL) is the most common inherited neurodegenerative disease in children, causing blindness, seizures, cognitive and motor decline, and premature death. We previously generated *Cln3*$^{ex7/8}$ knock-in mice, harboring the common ~1kb deletion mutation that gives rise to most cases of JNCL. Because JNCL specifically impacts neuronal function and survival, we have also established a neuron-derived cell culture model of JNCL, by conditional immortalization of cerebellar granule neurons from perinatal *Cln3*$^{ex7/8}$ knock-in mice (Cb*Cln3*$^{ex7/8}$ cerebellar cells). Utilizing these two genetically accurate systems, our studies reveal that general disruptions in membrane trafficking are a prevalent feature associated with the common JNCL mutation. In homozygous Cb*Cln3*$^{ex7/8}$ cerebellar neuronal precursors, we show altered endocytosis, altered lysosomal distribution, altered autophagic membrane trafficking, and mitochondrial dysfunction. Furthermore, our studies provide evidence that these disruptions occur early in the disease process, preceding accumulation of the mitochondrial ATPase, subunit c, a hallmark feature of JNCL. Genechip array analysis of 10-week homozygous *Cln3*$^{ex7/8}$ knock-in cerebellum versus wild-type littermate cerebellum complements these observations, revealing that a substantial number of genes whose protein products are involved in membrane trafficking and protein transport are differentially expressed. Other major categories of differentially expressed genes in 10-week homozygous *Cln3*$^{ex7/8}$ knock-in cerebellum are those involved in transcription, mitochondrial function, immune response, the ubiquitin pathway and cell signaling. Collectively, these studies in Cb*Cln3*$^{ex7/8}$ cerebellar precursor cells and *Cln3*$^{ex7/8}$ knock-in mice highlight pathways that merit further investigation to unravel the JNCL pathogenic process.

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Genotype/Phenotype Correlations in Pseudoxanthoma Elasticum (PXE). *P.F. Terry[1], S. Hariri[2], C. Moore[2], C.M. Vocke[1], L.G. Bercovitch[3,1], S.F. Terry[1], PXE International Genotype Consortium.* 1) PXE International, Washington, DC; 2) Centers for Disease Control and Prevention, Atlanta, GA; 3) Brown University, Providence, RI.

Purpose: To evaluate associations, if any, between PXE-related gene variants and phenotypes.

Materials and Methods: Using samples from 254 participants in the PXE International Blood and Tissue Bank/Genetic Alliance BioBank, PXE-related genotypes were detected in three independent laboratories with Transgenomics Wave technology utilizing DHPLC. Phenotypes, classified according the Phenodex indexing system criteria, were described for all study participants. Descriptive analysis was performed, and univariate and multivariate analysis were attempted on original data and after phenotypes were dichotomized. Additionally a subanalysis was performed on individuals over 40 years of age who are most likely to express all phenotypes of interest.

Results: Due to the high numbers of genetic variants and phenotype categories relative to the sample size, there was insufficient power to detect intended associations in this study.

Conclusions: Further analysis is needed to examine associations, not only between gene variants and clinical outcomes, but also to assess how these associations may be modified by environmental factors. In addition, analysis of correlations between mutations and cellular phenotype should be undertaken to identify effective treatment modalities.

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Late X-chromosome inactivation skewing of a NEMO mutation causes Incontinentia Pigmenti in a female patient with immunodeficiency: a new mutation in NEMO gene. *A. SMAHI[1], N. MARTINEZ[2], I. MUNOZ-SAA[2], D. HEINE-SUNER[3], A. MARTIN[4], A. MUNNICH[1], N. MATAMOROS[2].* 1) Département de Genetique INSERM U393, Hopital Necker, Paris, France; 2) Immunology Service, Hospital Universitario Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 3) Genetic Service, Hospital Universitario Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 4) Dermatology Service. Hospital Universitario Son Dureta, Palma de Mallorca, Balearic Islands, Spain.

Incontinentia pigmenti (IP) is an X-linked genodermatosis, lethal in males. Affected females survive because of X-chromosome dizygosity and the negative selection of cells carrying the mutant X-chromosome, which results in a skewed X-inactivation pattern. The most frequent mutation is a deletion of part of the NEMO gene (NEMO4-10), although others have been reported. Mutations of NEMO which do not totally abolish NF-B activity are consistent with male survival, and cause an allelic variant of IP called hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID). We present a de novo insA790 NEMO mutation in a female patient with non classical IP who also suffered from immunodeficiency because of a late and progressive selection against peripheral blood cells carrying mutated X-chromosome. At the age of three years and six months all immunodeficiency signs had disappeared, and the X-chromosome inactivation pattern was completely skewed. The low T cell proliferation and CD40L expression emphasize the important role of NEMO/ NF-B pathway in T cell homeostasis. The decreased amount of NEMO protein and the impaired IkB degradation suggest that this new mutation, may result in RNA or protein instability. This is, to our knowledge the first example of an in vivo selection against the mutated X-chromosome in a X-linked disease in human.

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Large deletion encompassing D4F104S1 in an extended pedigree with facioscapulohumeral muscular dystrophy.

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Facioscapulohumeral muscular dystrophy (FSHD) is a disease of skeletal muscle, with symptoms including both facial and shoulder girdle weakness. In the majority of FSHD cases, the molecular basis is a contraction of the D4Z4 repeat on the end of the long arm of chromosome 4. In a small number of cases, there is an apparent absence of the contracted D4Z4 repeat that is associated with FSHD. This is due to the presence of a deletion in the region proximal to the D4Z4 repeat array, which encompasses the p13E-11 (D4F104S1) probe-binding site. The frequency of such extended deletions is unknown, due to the difficulties in the molecular identification of such cases. To date, only a few patients with an extended deletion have been described, with no more than 2 members of the same family. Here we describe a family (DUK 2531) with 20 sampled individuals, where 10 affected individuals that segregate a contracted D4Z4 allele in addition to large proximal deletion of approximately 70 kb. This is the largest deletion to be described and the first extended pedigree showing such a deletion. This family was originally characterized as being unlinked to the 4q35 region (FSHD1B) and was thought to have normal D4Z4 fragment size. Because such deletions can lead to misinterpretation in the diagnostic setting, this suggests the need for the use of additional diagnostic tests in FSHD such as the 4qA probe. *Mse*I digestion and probing with the D4Z4 specific probe 9B6A reveals the presence of ten D4Z4 repeat units (33 kb), and the 4qA probe also reveals a fragment of this length. Subsequent *Mse*I/*Bln*I digestion confirms that the deletion allele is of chromosome 4 origin and *Not*I fragment analysis for chromosome assignment suggests that the allele resides on chromosome 4. It appears that the extended deletion includes the p13E-11 binding site, the B31 probe site, the inverted repeat D4S2463, and several genes including *FRG2* and *TUBB4Q*. Mapping of the exact breakpoint is currently underway.

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Pathogenesis of Cartilage Hair Hypoplasia. *P. Hermanns[1,4], A.A. Betruch[6], J.G. Leroy[1,2], T. Bertin[1], B. Dawson[1,5], A. Tran[1], E. Munivez[1,5], M. Schmitt[3], B.U. Zabel[4], B. Lee[1,5].* 1) Molecular & Human Genetics, Baylor Colllege of Medicine, Houston, TX, USA; 2) Ghent University Medical School, Ghent Belgium; 3) Biochemistry and Molecular Biology, Upstate Medical University Syracuse, NY, USA; 4) Children's Hospital, University of Mainz, Mainz, Germany; 5) Howard Hughes Medical Institute, Houston, TX, USA; 6) Pediatrics-Hematology & Oncology, Texas Childrens Feigin Centre, Houston, TX, USA.

CHH is an autosomal recessive disease characterized by dwarfism, fine & sparse hair, deficient cellular immunity and predisposition to malignancy. CHH is one of the few Mendelian diseases caused by mutation in a non-coding RNA gene, RMRP. It is the RNA component of a ribonucleoprotein complex. Yeast studies suggest its involvement in processing of pre-rRNA in the nucleolus, cleavage of mitochondrial RNA priming mitochondrial DNA replication, and cell cycle progression. Mutation analysis was performed in 34 patients, referred with the diagnosis of probable CHH. RMRP mutations were found in 26 subjects. The combination of reduced birth length, severe growth failure and deficient hair growth appeared to be highly correlated with the detection of mutations in the RMRP gene. So far no modifiers have been identified that could explain the pleitropic presentation of CHH. In vitro studies show that promoter duplications found in CHH patients cause a hypomorphic allele affecting RMRP transcription. Quantitative RT-PCR analysis of patient lymphoblasts revealed a 7-fold decrease in RMRP RNA level irrespective of the nature of mutations tested. RMRP mutations introduced into the yeast ortholog NME1 neither altered mitochondrial function nor, affected mitochondrial content in a CHH patient fibroblast cell line. The 70A>G causes an alteration in rRNA processing and microarray studies performed with two patients suggest that RMRP mutation is associated with significant up-regulation of several cytokines and cell cycle regulatory genes. These data suggest that alteration of ribosomal processing leads to altered cytokine signaling and cell cycle progression in terminally differentiated cell types involved in CHH pathogenesis, i.e., lymphocytic and chondrocytic lineages.

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Defective Protein Interactions as a Molecular Basis for Axenfeld-Rieger Syndrome (ARS). *B.A. Amendt[1], H.M. Espinoza[2], E.V. Semina[3], B.P. Brooks[4].* 1) Environmental and Genetic Medi, Texas A&M HSC, Houston, TX; 2) Department of Biological Sciences, University of Tulsa, Tulsa, OK; 3) Department of Medical Genetics, Medical College of Wisconsin, Milwaukee, WI; 4) National Eye Institute, National Institutes of Health, Bethesda, MD.

The PITX2 and Lymphoid Enhancer Factor (LEF-1) transcription factors are required for the inductive formation of several epithelial-derived organs including teeth. Individuals with Axenfeld-Rieger Syndrome present clinically with eye, dental and umbilical abnormalities as the hallmark of this genetic disorder. We are studying the transcriptional mechanisms of PITX2 and have identified several new PITX2 interacting factors, which regulate its transcriptional activity. PITX2 directly interacts with LEF-1 and -catenin to modulate gene expression. These interactions result in a synergistic activation of several gene promoters including LEF-1. A new C-terminal PITX2 mutation associated with ARS results in a deletion of a T nucleotide at position 1261 and changes the reading frame. Thus, this mutant protein (PITX2 T1261) is only 237 amino acids compared to 271 for wild type PITX2A. This mutation disrupts the distal C-terminal tail and OAR domain of PITX2. This mutant protein binds DNA normally, however it is unable to interact with several proteins including LEF-1. The inability of PITX2 T1261 to interact with LEF-1 severely decreases the transcriptional activation of this mutant protein compared to wild type PITX2. This mutation provides a functional analysis of PITX2 protein interactions in regulating normal human development. Protein interactions are required for the temporal and spatial regulation of transcription factors during embryogenesis and our data demonstrate a specific requirement for protein interactions in regulating PITX2 transcriptional activity. Thus, we have demonstrated a molecular basis for a genetic defect involving protein interactions.

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New mutations on FGFR 3 and FGFR2 genes responsible for classical Hypochondroplasia and Crouzon syndrome phenotypes. *S. Carlo[1], J. Acevedo[2], V. Franceschini[1], M. Lopez[2], A. Quintero-Del-Rio[1], N. Arciniegas[1], A.S. Cornier[1].* 1) Genetic Division, Ponce Sch Medicine, Ponce, PR; 2) Department of Nursing, Univ. of Puerto Rico, Arecibo, PR.

Mutations within the FGFR genes have been described as responsible for a wide variety of phenotypes. The FGFRs are a family of tyrosine kinase receptors with three immunoglobulin-like regions in the extracellular domain, a transmembrane domain, and a split intracellular tyrosine kinase domain. We are presenting a series of two patients with hypochondroplasia with a n unusual mutation in the FGFR 3 gene indicating the presence on a G>A transition in intron 11 responsible for hypochondroplasia phenotype that has not been reported in the literature before. A third patient with Crouzon syndrome classical phenotype and an Nt 1204 G>A mutation is described. None of these mutations /variations has been associated to complete phenotype in the literature before. Given that mutations responsible for genetic disorders in Puerto Rican (Hispanic) population is so limited we believe is of both clinical and molecular importance to recognize pathological molecular changes to provide the appropriate genetic counseling.

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Identification and functional characterization of a novel mutation in casein kinase I causing Familial Advanced Sleep Phase Syndrome. *Q.S. Padiath[1], Y. Xu[1], R.E. Shapiro[2], C.R. Jones[3], S.C. Wu[1], N. Saigoh[1], K. Saigoh[1], A. Shenoy[1], L.J. Ptacek[1,4], Y.H. Fu[1].* 1) Dept. of Neurology, UCSF, San Francisco, CA; 2) Dept. of Neurology, College of Medicine, University of Vermont, Burlington, VA; 3) Dept. of Neurology, University of Utah, Salt Lake City, UT; 4) HowardHughes Medical Institute, UCSF, San Francisco, CA.

Familial advanced sleep phase syndrome (FASPS) is a human behavioural phenotype characterized by early sleep times and early-morning awakening. It was the first human, mendelian circadian rhythm variant to be well-characterized, and was shown to result from a mutation in a phosphorylation site within the (CKI)-binding domain of the human PER2 gene. The identification of more FASPS families that did not carry the hPer2 mutation suggested the involvement of other genes producing this phenotype. We therefore set out to identify other mutations in human subjects leading to FASPS. We report here the identification of a missense mutation (T44A) in the human CKI gene, which results in FASPS. The mutation was not present in 250 normal controls and individuals with the mutation showed significantly earlier sleep and early-morning awakening times. This mutant kinase has decreased enzymatic activity in vitro. Transgenic *Drosophila* carrying the human CKI-T44A gene showed a phenotype with lengthened circadian period. In contrast, transgenic mice carrying the same mutation have a shorter circadian period. As some individuals with the mutation also presented with a history of depression behavioral analysis of the transgenic mice was also carried out.These results show that CKI is a central component in the mammalian clock, and suggest that mammalian and fly clocks might have different regulatory mechanisms despite the highly conserved nature of their individual components.

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Mutations of Connexin 43 (Cx43) Reveal a Range of Clinical Phenotypes and Functional Channel Effects. *W. Paznekas[1], H. Musa[2], A. Lai[3], J. Shibayama[2], D-N. Le[3], M. Delmar[2], A. Charles[3], E.W. Jabs[1].* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Depts Pharmacology & Microbiology/Immunology, SUNY, Syracuse, NY; 3) Dept Neurology, UCLA, Los Angeles, CA.

Cx43 mutations are found in patients with oculodentodigital dysplasia (ODDD), an autosomal dominant disorder characterized by craniofacial and limb anomalies and also affecting central nervous system, eye, skin, heart and hearing. Connexins form a hexameric cell membrane complex known as a connexon or hemichannel. Coupling of connexons from adjacent cells forms a gap junction (GJ), an intercellular channel of low resistance between cytoplasmic compartments. Known Cx43 mutations total 37 (5 recurrent), occur in most regions of the protein, and result in phenotype ranging from syndactyly type III alone to an autosomal recessive case with features of both ODDD and Hallermann-Streiff syndromes. For channel studies we selected ODDD mutations Y17S, G21R, A40V, F52dup, L90V, I130T, K134E, & R202H for their associated phenotypic features and affected protein domain. Transfection studies of mutant Cx43 in GJ communication deficient HeLa showed reduced GJ plaque formation for all mutants compared to Cx43WT, with F52dup & R202H failing to form plaques. In transfected mouse N2A neuroblastoma electrical conductance of mutant homotypic channels relative to WT is reduced or lacking. Heterologous channels were formed by co-expression of Cx43WT with mutants F52dup or R202H, which were unable to form homotypic GJ plaques. Junctional conductance of cells co-expressing mutant to WT (1:1) showed a dominant negative effect for L90V but with F52dup or R202H conductance was not altered until higher ratio of mutant to WT. Mutants Y17S, L90V, I130T associated with neurological dysfunction and G21R, A40V, F52dup without neurological findings were tested in rat C6 glioma (low expressers of Cx43). All mutants could be detected on the C6 cell surface though GJ clusters for F52dup were fewer. Hemichannel function (indicated by propidium iodide uptake) and GJ dye transfer (by scrape-loading assay) were examined for mutants compared to WT. Thus, both hemichannel and gap junction function were impaired for all ODDD mutants tested.

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Custom array comparative genomic hybridization applications in Pelizaeus-Merzbacher Disease. *J.A. Lee[1], K. Inoue[2], J.R. Lupski[1,3,4].* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, Texas; 2) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; 3) Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 4) Texas Childrens Hospital, Houston, Texas.

Pelizaeus-Merzbacher Disease (PMD) is a rare X-linked genomic disorder characterized by dysmyelination in the central nervous system. A majority of cases are caused by dosage alterations of the proteolipid protein 1 gene *PLP1* on chromosome Xq22, including *PLP1* duplication in 60-70% of cases, and also rare cases of *PLP1* gene deletion. The remaining 10-20% of PMD cases are associated with point mutations. We developed a custom array comparative genomic hybridization (aCGH) assay, including 68 overlapping large-insert bacterial and P1 artificial chromosome clones tiling an approximate 10 Megabase region including and surrounding the *PLP1* gene. Therefore, our custom assay will detect dosage alterations within the region tested, a region which encompasses the causative genomic rearrangement in nearly all PMD duplication and deletion cases examined. Importantly, our aCGH assay can be applied to perform rearrangement breakpoint analysis, to determine the actual sizes of the duplications and deletions, and also for prenatal diagnosis of PMD caused by *PLP1* dosage alteration.

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Complex rearrangements in Pelizaeus-Merzbacher disease. *G.M. Hobson[1,2], K. McLean[1], K. Sperle[1].* 1) Nemours Biomedical Research, A I duPont Hosp Children, Wilmington, DE; 2) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

Duplication of a genomic region that includes the proteolipid protein gene (*PLP1*) is the most common mutation causing Pelizaeus-Merzbacher disease (PMD), a dysmyelinating disorder of the central nervous system. Although families have been reported in which the additional *PLP1* genomic region has integrated into a location that is distant from the normal *PLP1* locus at Xq22, most duplications arise by intrachromosomal recombination and have a tandem head-to-tail orientation. Our studies using quantitative multiplex PCR map the extent of duplication in PMD patients with respect to the reference genomic sequence available at www.ensembl.org. In 4 of 36 patients examined, we have found a second duplicated genomic region in addition to the PLP1-containing duplication. In 2 of these patients, duplicated regions of at least 37 and 436 kb map proximal of the *PLP1*-containing duplications. The proximal end of one of these duplicated regions and the distal end of the other map within the same 26 kb interval. In the other two patients, duplicated regions of at least 15 and 20 kb map distal of the *PLP1*-containing duplications in the low copy repeat (LCR) region. Our data support the notion that genomic structure in the area of the *PLP1* gene can lead to complex rearrangements.

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Gr/gr deletions within the AZFc region of the human Y chromosome may not be associated with spermatogenic failure. *S. Chantot-Bastaraud[1, 2], C. Ravel[1, 2], B. El Houate[2], G. Vinci[2], J. Mandelbaum[1], J.P. Siffroi[1], K. McElreavey[2].*
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Three regions of the long arm of the Y chromosome, termed AZFa, AZFb and AZFc (AZF: azoospermia factor) are associated with spermatogenic failure. AZFc is the most commonly deleted interval in men with azoospermia or severe oligozoospermia (<1x106/ml)and it is in a region of the Y chromosome that is made up almost entirely of long direct and inverted repeats. Recently a 1,6 Mb deletion termed gr/gr was identified in infertile men with no other known cause of male infertility. This deletion removes the proximal portion of AZFc. The gr/gr deletion is therefore considered a significant risk factor for reduced sperm counts leading to male infertility. Here, we performed a case/control study to determine if the gr/gr deletion is associated with reduced sperm counts in a population consisting of men attending an infertility clinical with idiopathic infertility. In the infertile population we found that 4/192 individuals carried a gr/gr deletion. In the control population we found that 6/181 men carried a gr/gr deletion. For each gr/gr deleted chromosome, the Y haplotype was determined using binary markers. The results indicated that gr/gr deletion has arisen independently on several Y chromosome lineages. A trend is indicated by the distribution of the Y haplogroups between the control group and individuals with spermatogenic failure. All normospermic individuals belong to either the Y chromosome lineage E3b2 or P(xR1a), whereas the patients with spermatogenic failure fall within other Y chromosome lineages. This suggests that some Y chromosome lineages may protect against the effects of the gr/gr deletion, or that the type of gr/gr deletion may differ between lineages and have variable effects on spermatogenesis. We recommend that screening for gr/gr deletions in a clinical setting should be used with caution until larger studies are conducted on men from different ethnic groups and geographical regions.

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Copy number alterations of TEKT3 in Charcot-Marie-Tooth disease. *F. Baas[1], M.A.J. Weterman[1], F. van Ruissen[1], M. de Wissel[1], L. Bordewijk[1], J.P.A. Samijn[2], J. Hoogendijk[3], F. Meggouh[1].* 1) Neurogenetics Lab, Academic Medical Center, Amsterdam, Netherlands; 2) Medisch Centrum Rijnmond Zuid 3075 EA Rotterdam, Netherlands; 3) Dept of Neurology, Rudolf Magnus Institute of Neuroscience, UMCU, Utrecht, Netherlands.

Charcot-Marie-Tooth disease is a genetically heterogeneous disorder. To date, more then 20 genes have been implicated in the disease process, and many other CMT loci have not yet been linked to a disease gene. Nerve conduction velocities are used to distinguish between diseases with demyelinating or axonal features. In demyelinating CMT the primary defect is in the formation or maintenance of a normal myelin sheet around the axon. The most frequent form of demyelinating CMT (CMT1a) is linked to chromosome 17 (17p11.2). Duplications and mutations of the PMP22 gene are found in the majority of CMT1a patients. Here we describe several apparently unrelated individuals with CMT1 who carry a copy number polymorphism (CNP) centromeric of PMP22. These CNPs do not include PMP22 itself. We propose that the CNP is responsible for a rare form of CMT1a, since this CNP is thus far only found in CMT patients in whom alterations of PMP22 were excluded. Most diagnostic tests for CMT1a will not detect this CNP.

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Duchenne muscular dystrophy in a phenotypic female with androgen insensitivity: A case report. *J. Sullivan, C. Nichter, T. Marini, H. Allen, S. Pflueger.* Baystate Medical Center and Children's Hospital and Tufts University School of Medicine, Springfield, MA.

We report an unusual case of Duchenne muscular dystrophy (DMD) in a phenotypic female. At delivery the infant presented with clitoromegaly and failure to thrive. Female gender was assigned. Subsequent cytogenetic evaluation revealed a 46,XY karyotype. Endocrinology evaluation was consistent with a diagnosis of partial androgen insensitivity (PAIS). At age 5 the child was referred to neurology for proximal muscle weakness. Reported maternal family history raised the question of a male relative with some form of muscular dystrophy. Molecular testing revealed a deletion of exons 50-52 of the dystrophin gene, consistent with a clinical diagnosis of Duchenne muscular dystrophy. Molecular testing for AIS identified a hemizygous G623A missense mutation in exon 3. Although this mutation has not been previously reported, this is the likely explanation for the female phenotype in a karyotypic male. The male karyotype explains the expression of the X-linked disorder in this female child. Patients with Turner syndrome (monosomy X) may present with unexpected X-linked traits, however in the absence of stigmata of Turner syndrome the possibility of androgen insensitivity should be considered in the differential diagnosis of an X-linked condition in a phenotypic female.

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Early transposable element intron insertion results in aberrant transcription of the *Hsf4* gene and cataracts in *lop11* mice. *D. Sidjanin[1], E. Talamas[1], L. Jackson[1], M. Koeberl[1], M. Jablonski[2], B. Chang[3].* 1) Dept Ophthalmology, Medical Col Wisconsin, Milwaukee, WI; 2) Dept Ophthalmology, University of Tennessee Memphis, TN; 3) Jackson Laboratories, Ben Harbor, ME.

Lens opacity 11 (*lop11*) is an autosomal recessive mouse cataract mutation that arose spontaneously in RIIIS/J strain. At weaning, mice exhibit total cataracts with vacuoles. In order to map this locus, *lop11* mice were outcrossed to CAST/Ei and F1 mice were backcrossed to *lop11* to generate 195 backcross progeny. The progeny were evaluated at weaning with a slit lamp following mydriasis with atropine, euthanized and tissues were collected. Genomic DNA was isolated from collected spleens. Polymorphic microsatellite markers were selected to evenly cover the mouse genome. Linkage was detected between *lop11* locus and microsatellite markers on chromosome 8. No recombinants were detected with *D8Mit198*. Evaluation of a mouse map of the *lop11* critical region identified *Hsf4* as a candidate gene. In humans, mutations in *HSF4* lead to development of cataracts. Exon scanning of all 13 *Hsf4* exons did not reveal any sequence difference between *lop11* and wild type. Further analysis identified a 5.5 kb insertion of an early transposable element in intron 9 about 60 bp upstream from intron/exon junction. Northern blot from *lop11* eye tissues hybridized with an *Hsf4* probe from exons 10-13 did not identify any *Hsf4* transcripts in *lop11*. The same blots when hybridized with an *Hsf4* probe from exons 1-6 revealed at least 100 fold up-regulated *Hsf4* transcript in *lop11*. These results suggest that in *lop11* mice an early transposable element insertion in intron 9 alters splicing and expression of the *Hsf4* gene. The *lop11* mouse is an excellent animal model for cataractogenesis in patients with mutations in human *HSF4* gene.

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A *VCAM1* Promoter Mutation That Affects Transcription Factor Binding is Observed with Increased Prevalence in Adults with Sickle Cell Anemia. *J.G. Taylor[1], R.A. Chen[2], C. Haudenschild[1], N. Ragavachari[1], T. Bernig[2], D. Garcia-Rossi[3,4], A. Hutchinson[3,4], C. Hoppe[5], M.T. Gladwin[1], S.J. Chanock[2,4].* 1) Vascular Therapeutics Section, NHLBI, NIH, Bethesda, MD; 2) Section on Genomic Variation, NCI, NIH, Gaithersburg, MD; 3) SAIC, NCI-FCRDC, Frederick, MD; 4) NCI Core Genotyping Facility, Gaithersburg, MD; 5) Children's Hospital, Oakland, CA.

Sickle cell anemia (SCA) is characterized by a wide phenotypic spectrum of complications that are hypothesized to be partly attributable to inter-individual variation in modifier genes. Common *VCAM1* promoter SNPs have been associated with susceptibility to SCA complications including strokes. To refine these associations, we identified variants in healthy controls and then used phylogenetic analysis to prioritize promoter haplotypes for further study. Using comparative genomic alignments, a singleton promoter haplotype defined by 2 SNPs within phylogenetically conserved regions including a site at -540 was selected for functional analysis. In vitro, this promoter haplotype has higher reporter gene transcription in response to cytokine stimulation relative to wild type (3.4 fold and 1.8 fold, respectively, P = 0.01). Real time PCR in a cell line with the -540 genotype correlates with the in vitro data. Putative transcription factor (TF) binding sites are present at both SNP sites, although electromobility shift assays only demonstrate allele specific binding of c/EBP TF family members at -540 based upon preliminary study. In humans, the -540 genotype is carried by 3.7% of 81 adults with homozygous SCA while it is present as a mutation in only 1 of 255 African American controls (0.4%; P = 0.045), suggesting that this variant could be associated with a functional role in SCA. Studies are underway in larger populations to confirm this observation, and to determine the incidence of this variant in newborns with SCA. Finally, this variant represents a true mutation as only 1 additional heterozygote was identified in a survey of 2234 chromosomes from worldwide populations. Taken together, these detailed studies suggest that analysis of both common and rare variants will be required to identify genetic modifiers of monogenic diseases like SCA.

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Townes-Brocks syndrome: 25 novel mutations in sporadic and familial cases and update on the mutational spectrum. *E.M. Botzenhart, B. Roesler, J. Kohlhase, Institute for Human Genetics and Anthropology, University of Freiburg.* Institute for Human Genetics and Anthropology, Freiburg University, Freiburg, Germany.

Townes-Brocks syndrome (TBS; OMIM#104780) is a rare autosomal dominantly inherited malformation syndrome due to mutations in SALL1, a human gene related to the developmental regulator SAL of Drosophila melanogaster. The clinical presentation of TBS is highly variable. It is predominantly characterized by renal, anal, ear and limb anomalies. Different types of hearing loss, urogenital malformations cardiac malformations, and mental retardation may additionally occur. The SALL1 gene is coding for a zinc finger protein thought to act as a transcriptional repressor. It is composed of four highly conserved evenly distributed double zinc finger domains and a C2HC motif at the amino terminus. A single C2H2 motif is attached to the second double zinc finger domain. Initially SALL1 mutations have been postulated to cause TBS by haploinsufficiency. A null allele of mouse SALL1 however does not mimick the human syndrome. Another mouse model, mimicking a typical TBS causing mutation showed that TBS mutations lead to the formation of truncated proteins with dominant negative effect in mice thereby causing the disease. Further research into the molecular mechanisms of these mutations will however be necessary to demonstrate the functional consequences of SALL1 mutations in humans. To date 23 SALL1 mutations leading to TBS have been described. Nearly all of these with the exception of R276X occur only in single patients or families thereby preventing phenotype-genotype correlations. Here we present 25 novel mutations associated with TBS in 26 unrelated families increasing the number of SALL1 mutations to 48.

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Novel Mutations of the NIPBL gene in Cornelia de Lange Syndrome. *M. ZARHRATE[1], L. COLLEAUX[1], A. david[2], A. MUNNICH[1], V. CORMIER DAIRE[1], J. BONNEFONT[1].* 1) INSERM U393 and Department of Genetics, Hospital Necker Enfants Malades, Paris, France; 2) Genetics unit Hopital Mere-Enfant, Nantes, France.

Cornelia de Lange syndrome (CdLS) is characterised by facial dysmorphism, microcephaly, growth and mental retardation, and congenital anomalies including limb defects. Mutations in the gene NIPBL, the human homolog of Drosophila Nipped-B, have recently been found in approximately 50% of CdLS cases. We present here the molecular analysis in a series of thirteen children with CdLS. All children presented with typical features of CdLS but none of them had major limb anomalies. Fifty-one primer pairs were designed to cover all 46 NIPBL coding exons and the corresponding exon-intron boundaries. Direct sequencing enabled to identify three heterozygous de novo NIPBL mutations including two missense [c.7189T>C (p.S2397P), c.6166C>T (p.P2056S) and one splice site (64+5G>A) mutations. These mutations were not found in 100 control chromosomes. Additionally, analysing segregation of 3 poly (AC) intragenic markers ruled out large deletions in the remaining patients. Thus, NIPBL mutations account for only 1/4 of CdLS cases in our series. The absence of NIPBL mutation in more than 50% of CdLS individuals in the few series reported so far may suggest either the limitation of the screening methodology or the genetic heterogeneity of the disorder.

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Residual splicing of the major Shwachman-Diamond syndrome allele with a non-canonical splice donor-acceptor pair. *L.L. Chen[1,2], G.R.B. Boocock[1,2], J.M. Rommens[1,2].* 1) Department of Molecular & Medical Genetics, University of Toronto; 2) Program in Genetics & Genomic Biology, The Hospital for Sick Children, 555 University Ave, Toronto, ON CANADA M5G 1X8.

Shwachman-Diamond Syndrome (SDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, skeletal abnormalities, bone marrow failure and predisposition to hematologic malignancies. SDS is caused by mutations in the SBDS gene, which is a member of a highly conserved protein family thought to be involved in RNA processing and/or ribosome biogenesis. One of the two common mutations found in SDS patients involves a donor splice site, 258+2TC, leading to a non-canonical GC-AG donor-acceptor pair for intron 2. The non-canonical donor-acceptor pair has been observed in less than 0.7% of all human exons. RT-PCR analysis of patient RNA indicated that the mutant allele uses an upstream cryptic donor splice site in exon 2 that leads to frameshift and a truncated SBDS protein. The second common mutation, 183-184TACT, also predicts a stop codon and truncated protein. The frequent occurrence of patients that are compound heterozygotes for the common mutations (102/257 families) and the absence of homozygotes with the 183-184TACT mutation (0/257 families), together with the essential nature of SBDS in mice, indicate that the 258+2TC allele is hypomorphic and should lead to residual SBDS activity. To examine the associated mechanism, an expression vector containing a complete and tagged SBDS gene has been constructed. Transfection of human cell lines indicated that very low levels of SBDS are expressed from the mutated allele. The level of residual protein varied by cell type and could be increased by removal of the upstream cryptic donor splice site in exon 2. Since the majority of unrelated SDS patients (174/257) possess at least one allele with this mutation, therapeutic benefit may be realized by determining which factors and cell conditions contribute to the use of the non-canonical splice donor-acceptor GC-AG pair.

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Nonsense-mediated decay (NMD) might be involved in phenotypic heterogeneity of *ROR2* mutations. *J. Lupski[1, 2, 3], S. Ben-Shachar[1], M. Khajavi[1], U. Cheema[1], N. Zamani[1], H. van Bokhoven[4].* 1) Department of Molecular & Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Childrens Hospital, Houston, Texas; 4) Department of Human Genetics, Radboud University Nijmegen Medical Centre The Netherlands.

Mutations in *ROR2*, encoding a receptor tyrosine kinase, can cause the autosomal recessive form of Robinow syndrome (RRS, OMIM# 268310), a severe skeletal dysplasia with shortening of the limbs, segmental defects of the spine, brachydactyly, and a dysmorphic facial appearance. Various nonsense and frameshift mutations in ROR2 result in both RRS and a dominant form of Brachydactyly type B (BDB, OMIM #113000), but no functional mechanisms have been delineated to effectively explain the association between mutation and different mode of inheritance causing different phenotypes (i.e. genotype/phenotype correlations). Interestingly, most mutations in ROR2 that results in premature termination codons (PTCs) in downstream exons appear to result in BDB segregating as an autosomal dominant trait whereas PTCs in upstream exons result in RRS. Previous studies have revealed decreased *ROR2* mRNA levels in fibroblast cell lines of patients with PTCs causing the autosomal recessive RRS. Considering the location of truncation in the gene, here we are investigating whether mutations that could possibly trigger the nonsense mediated decay (NMD) pathway and therefore decrease mRNA levels can cause a different phenotype with a distinct mode of inheritance. Although in theory all nonsense and frameshift mutations resulting in PTC are natural targets of NMD RNA surveillance system, it is not certain if NMD is the only pathway actually responsible for modulating disease severity by altered mRNA stability. To address this hypothesis, we have selected cell lines from patients carrying PTC mutations and are studying the potential role for NMD in the abrogation of aberrant effects of selected mutant alleles.

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Nephrogenic Diabetes Insipidus in Mice Caused by Deleting C-terminal Tail of Aqp2. *B. Yang, P. Shi, E. Sweezer, S. Guo, T. Kinney, A. Assad, K. Huffman, X. Cao.* Dept OB/GYN, Univ Iowa, Iowa City, IA.

In mammals, the tight hormonal regulation of water homeostasis is mediated by the aquaporin-2 water channel (AQP2) of the collecting duct (CD). Vasopressin induces redistribution of AQP2 from intracellular vesicles to the apical membrane of CD principal cells, accompanied by increased water permeability. Mutations of *AQP2* gene cause recessive and dominant nephrogenic diabetes insipidus (NDI), a disease in which the kidney is unable to concentrate urine in response to vasopressin. In mouse complete inactivation of this gene resulted in neonatal lethality due to fluid loss. In this study, we generated a line of mice with the distal C-terminal tail of the Aqp2 deleted ($Aqp2^{231}$), including the protein kinase A phosphorylation site (Ser-256), while still retaining the putative apical targeting signal (221-230) at the C-terminus. Mice heterozygous for the truncation appear normal. Homozygotes are viable to adulthood, with reduced urine concentrating ability: increased urine output (5-10 times more than control mice) and decreased urine osmolality (193 17 in $Aqp2^{231/231}$ vs 1,173 130 mOsm/kg in control mice), and increased daily water consumption. While desmopressin can increase urine osmolality in wild type mice, it has no effect on $Aqp2^{231/231}$ mice, confirming that Aqp2 is regulated by hormone-activated protein kinase A phosphorylation of Ser-256 in the C-terminal cytoplasmic domain of the protein. Kidneys from affected mice showed CD and pelvis dilatation and papillary atrophy due to polyuria. Immunohistolological analyses using antibody against the C-terminal region of the protein could not detect any channels in the affected kidney, while antibody against the N-terminal region of the protein showed CD-specific staining, confirming the deletion of the C-terminal tail of Aqp2. CD-specific staining was obtained in wild type kidney using both antibodies. Thus we have generated a mouse model of NDI and it should be useful in studying the physiology and potential therapy of this disease.

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Association of dystrophin Dp71 with the nuclear matrix of PC12 cells. *R. Rodriguez-Munoz[1], M. Villarreal-Silva[1], F. Garcia-Sierra[2], B. Cisneros[1].* 1) Dept Genetics & Molec Biol,; 2) Dept Cell Biol, CINVESTAV-IPN, Mexico.

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by mutations in the dystrophin gene. Dystrophin Dp71 is the major product of this gene in the brain, but its function is poorly understood. Interestingly, C-terminal mutations of the DMD gene that would adversely affect the expression of Dp71 are associated with mental retardation. To study the function of Dp71 in neuronal cells, we previously adopted the PC12 neuronal cell line as a model. Recently, our group determined that alternative splicing regulates the cytoplasmic or nuclear localization of Dp71. We believe that the nuclear localization of Dp71 is a new and interesting clue for understanding its cellular function. It is thought that the nuclear matrix constitutes the nucleoskeleton structure that supports and modulates the main nuclear functions, such as DNA replication, transcription, and RNA splicing. Therefore, in this study we analyzed the possible association of Dp71 with the nuclear matrix of PC12 cells. By Western blot analysis of nuclear matrix preparations, we determined that Dp71 is a component of the PC12 nuclear matrix. By immunofluorescence analysis of in situ nuclear matrix preparation we observed that Dp71 localizes in the periphery and inner part of the nuclear matrix of undifferentiated PC12 cells. Interestingly, in the NGF-induced differentiated PC12 cells, the immunostaining of Dp71 accumulates mainly in the nuclear matrix periphery; additionally, we noted a labeling in a nucleoli-like structure. To analyze the nature of the association of Dp71 with the nuclear matrix, we withdrawn the internal nuclear matrix components with RNase A (300 g/ml) treatment followed by 650 mM (NH4)2SO4 and 20 mM DTT; such treatment resulted in a dramatic Dp71 immunostaining decrease in the internal nuclear matrix, but the Dp71 located in the nuclear matrix periphery remained unaltered. These results constitute the first evidence implicating Dp71 in the organization of the nuclear matrix.

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Functional analysis of PTPN11/SHP-2 mutants identified in Noonan syndrome and childhood leukemia. *T. Niihori[1], Y. Aoki[1], H. Ohashi[2], K. Kurosawa[3], T. Kondoh[4], Y. Narum[1], Y. Suzuki[1], S. Kure[1], K. Fujii[5], M. Imaizumi[6], Y. Matsubara[1].* 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Miyagi, Japan; 2) Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan; 3) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 4) Department of Pediatrics, Nagasaki University of Medicine, Nagasaki, Japan; 5) Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan; 6) Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, Japan.

Noonan syndrome (NS) is characterized by short stature, characteristic facial appearance, and'@heart defects. Recently, missense mutations of tyrosine phosphatase SHP-2 were identified in patients with NS and childhood leukemia. It remains unknown whether functional differences existed between the mutations identified in NS and leukemia.'@In this study, we identified ten mutations including a novel F71I mutation in 17 of 41 NS patients, and two mutations in 3 of 29 patients with leukemia. Immune complex phosphatase assays of individual mutants transfected in COS7 cells showed that ten mutants identified in NS and four mutants in leukemia showed 1.4-fold to 12.7-fold increased activation compared with wild-type SHP-2. These results suggest that the pathogenesis of NS and leukemia is associated with enhanced phosphatase activity of mutant SHP-2. A comparison of the phosphatase activity in each mutant and a review of previously reported cases showed that high phosphatase activity observed in mutations at codons 61, 71, 72, and 76 was significantly associated with leukemogenesis. Furthermore, we directly examined the phosphatase activity of SHP-2 in the lymphoblastoid cell lines from NS patients. This assay would provide an alternative method for diagnosing NS.

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A TNNI2 mutation in a family with Distal Arthrogryposis type 2B. *J. Hoo[1], A. Shrimpton[1, 2].* 1) Dept Pediatrics, SUNY Upstate Med Univ, Syracuse, NY; 2) Dept Pathology, SUNY Upstate Med Univ, Syracuse, NY.

Linkage mapping in a three-generation family with a distal arthrogryposis (DA) phenotype intermediate between DA1 and DA2A indicated linkage to 11p15.5 (sites of TNNI2 and TNNT3 genes) but not 9p13 (site of TPM2 gene). Follow up DNA sequencing of the TNNI2 gene (troponin subunit I [inhibitory] of fast-twitching skeletal muscle isoform) detected a three base pair deletion that would be predicted to result in the deletion of a glutamic acid at codon position 167 (delE167). This mutation, like the two previously described TNNI2 mutations, is located in the carboxy-terminal domain and thus supports the existence of a TNNI2 critical region sensitive to alteration that will give rise to distal arthrogryposis. Physical examination of family members confirms the high degree of variability in expression amongst mutation carriers.

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Mutations of the CYP21 gene among Filipino patients with Congenital Adrenal Hyperplasia. *E. Cutiongco[1], K. Goji[2], M. Matsuo[3].* 1) Inst Human Genetics, National Inst Health Philipine, Metro Manila, Philippines; 2) Department of Endocrinology and Metabolism, Kobe Children's Hospital, Japan; 3) Department of Pediatrics, Kobe University Graduate School of Medicine, Japan.

Congenital Adrenal Hyperplasia (CAH), an autosomal recessive disorder, is due to defective enzymes involved in adrenal steroidogenesis. Phenotypic manifestations are variable depending on the effects produced by the deficient hormones and by the excess production of steroids unaffected by the enzymatic block. The worldwide incidence of CAH is 1 in 15,000 with ethnic and racial variability. The incidence in France, Italy, Scotland, New Zealand and Japan ranges from 1 in 10,000 to 1 in 23,000. Among Filipinos, the crude incidence of CAH is 1 in 6,023 (Philippine Newborn Screening), which is higher than what is reported in most populations. More than 90 percent of cases result from a 21-hydroxylase (cytochrome p450c21) deficiency involving two 21-hydroxylase genes CYP21, the active gene, and CYP21P, a pseudogene. Studies have shown that mutations result from unequal crossover during meiosis, which leads to complete deletion of the gene, gene conversion events or to point mutations. Majority of these studies have demonstrated differences in the frequency of several gene mutations. Direct DNA sequencing of the CYP21 gene was performed among Filipino CAH patients and their families. In this study, the majority of cases, 11/13 or 85 percent; had a detectable mutation in the CYP21 gene. The mutation most frequently found in these families was the nucleotide 656 intron 2, a premature splicing error. However, in 2/13 or 15 percent; of the cases, a mutation could not be found. The determination of the most frequent alleles in our population will facilitate rapid screening for detection of mutations in the 21-hydroxylase gene. Establishment of a definitive diagnosis can also be made available which are important in the management and counseling of Filipino CAH cases.

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Unregulated insulin secretion by pancreatic beta-cells in hyperinsulinism/hyperammonemia syndrome: Role of glutamate dehydrogenase, ATP-sensitive potassium channel, and nonselective cation channel. *Y. Okano[1], M. Kawajiri[1], M. Kuno[2], D. Tokuhara[1], Y. Hase[3], H. Inada[1], F. Tashiro[4], J. Miyazaki[4], T. Yamano[1].* 1) Dept Pediatrics, Osaka City Univ Grad Sch of Med, Osaka, Japan; 2) Dept Physiology, Osaka City Univ Grad Sch of Med, Osaka, Japan; 3) Osaka City Environment and Public Health Center, Osaka, Japan,; 4) Div Stem Cell Regulation Res, Osaka Univ Grad Sch of Med, Osaka,Japan.

The hyperinsulinism/hyperammonemia syndrome is caused by 'ggain of function'h of glutamate dehydrogenase (GDH). Several missence mutations have been found; however, cell behaviors triggered by the excessive GDH activity have not been fully demonstrated. This study was aimed to clarify electrophysiological mechanisms underlying the dysregulated insulin secretion in pancreatic beta-cells with GDH mutations. GDH kinetics and insulin secretion were measured in MIN6 cells overexpressing the mutant GDH constructs, site-1 (G446D) and site-3 (L413V) mutations. Membrane potentials and channel activities were recorded under the perforated-patch configuration that preserved intracellular environments during recordings. In mutant MIN6 cells, sensitivity of GDH to GTP was reduced and insulin secretion at low glucose concentrations was enhanced. The basal GDH activity was greatly elevated in L413V bearing a mutation in the antenna-like structure (site-3). The L413V cells were depolarized greatly without glucose, often accompanying by repetitive Ca^{2+} firings. Intracellular dialysis with the ATP-free solution hyperpolarized cells. The KATP channel activity was suppressed and the nonselective cation channel (NSCC) activity was potentiated, while sensitivity of the channels to their specific blockers or agonists was not impaired. The site-3 GDH mutation increased the intracellular ATP/ADP ratio, which in turn caused sustained depolarization not only by closure of the K_{ATP} channel, but also by opening of the NSCC. The resultant activation of the voltage-gated Ca^{2+} channel appears to induce hyperinsulinism. The present study provides evidence that multiple channels co-operate in unregulated insulin secretion in pancreatic beta-cells of the hyperinsulinism/hyperammonemia syndrome.

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Homodimerization of DAX1: Complexity in Molecular Pathogenesis of Adrenal Hypoplasia Congenita. *A.K. Iyer[1], E.R.B. McCabe[1,2].* 1) Human Genetics and; 2) Pediatrics, UCLA David Geffen Sch of Med, LA, CA 90095-1752.

Adrenal Hypoplasia Congenita (AHC) is caused by mutations in *NR0B1* encoding the nuclear receptor DAX1. AHC is also caused by mutations in *NR5A1* that encodes SF1. DAX1 functions as an SF1 antagonist, and it is a conundrum how mutations in SF1, or its repressor DAX1, lead to similar phenotypes, and suggests additional complexity in AHC pathogenesis. Nuclear receptors function as monomers, homodimers or heterodimers. Our purpose was to determine if DAX1 forms a homodimer. A DAX1 homodimer was detected using the yeast two-hybrid (Y2H) system, *in vitro* co-immunoprecipitation (Co-IP), and Co-IP in mammalian cells. In Y2H, cotransformation of GAL4DB-DAX1 (GAL4 DNA-binding domain fused to DAX1) and GAL4AD-DAX1 (GAL4 Activation Domain fused to DAX1), resulted in activation of the HIS3 and -galactosidase reporter genes, providing evidence of an interaction; each construct alone did not activate the reporter. Homodimerization was confirmed *in vitro* using Co-IP with *in vitro* translated epitope tagged proteins. Using a myc antibody, HA-DAX1 was co-immunoprecipitated in the presence but not the absence of myc-DAX1. The presence of the DAX1 homodimer was further confirmed in HEK293 and HeLa cells through Co-IP using FLAG- and myc-tagged DAX1. Using a FLAG antibody, myc-DAX1 was co-immunoprecipitated in the presence but not in the absence of FLAG-DAX1. DAX1 localized to the nuclear and cytoplasmic compartments in HEK293 and HeLa cells, and Co-IP experiments with nuclear and cytoplasmic fractions showed that myc-DAX1 co-immunoprecipitated with FLAG-DAX1 in both compartments. A Co-IP based protein interaction competition assay demonstrated a decrease in DAX1 homodimer formation in the presence of increasing amounts of SF1, suggesting that the DAX1 homodimer dissociated to form heterodimers with SF1. We conclude that DAX1 homodimers exist in mammalian cells in the cytoplasmic and nuclear compartments. We speculate that the homodimer functions as a holding pool for DAX1 when not complexed with SF1. DAX1 homodimerization suggests the possibility of a novel function independent of its coregulator role, and additional complexity in the pathogenesis of AHC.

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Analysis of the *SLC6A19* D173N allele in Hartnup Disease. *D.N. Azmanov[1], H.J. Rodgers[1], C. Bailey[2], S. Bröer[3], J.E.R Rasko[2], J.A. Cavanaugh[1].* 1) Medical Genetics Research Unit, ANU, Canberra, Australia; 2) Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney, Australia; 3) School of Biochemistry & Molecular Biology, ANU, Canberra, Australia.

We recently identified mutations in *SLC6A19* as the cause of the underlying defect in neutral amino acid transport in Hartnup Disease (1). The D173N mutation displays reduced function *in vitro* and is the most frequent disease associated mutation. We have determined the haplotype surrounding this mutation which has a common origin in all individuals studied to date and estimated the age of this mutation in the Australian population of Caucasian descent. A combination of two microsatellites and seven SNPs covering 200kb were genotyped in a total of 41 individuals. Of these, 23 unrelated individuals carry the D173N mutation, four of whom are unaffected heterozygotes from the normal population. Eight unrelated individuals from the remaining 19 D173N positive individuals were from 7 affected families in which the D173N allele is segregating. Thus 44 D173N negative chromosomes were also genotyped.

We used three different methods to achieve estimates of the age of the D173N allele as follows: 36-81 generations using Bayesian linkage disequilibrium estimation (2); 41 generations (variance 23-205) intra-allelic variability estimation (3); 50.6 generations (variance 29.6-176) inter-haplotype variation (4).

We observed unusually long-range extended haplotype homozygosity in the D173N chromosomes in comparison to normal chromosomes ($p<0.0001$). We estimate the age of this allele to be 1000 - 1500 years, placing its likely origin in Europe and postulate that the survival of the allele may be a consequence of its incomplete functional inactivation and/or positive natural selection.

References:

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Gene-dosage influences the age at onset of SCA2. *P. Spadafora[1], N. Cutuli[2], P. Tarantino[1], S. Carrideo[1], I.C. Candiano[1], E.V. De Marco[1], D. Civitelli[1], F.E. Rocca[1], F. Annesi[1], V. Giuffrida[2], G. Annesi[1].* 1) Inst. of Neurological Sciences, National Research Council, Mangone, (CS), Italy; 2) Institute of Neurology, University of Catania, Italy.

Spinocerebellar ataxia type2 is the most frequent cause of autosomal dominant cerebellar ataxia type1 in Southern Italy. Clinically SCA2 is characterized by gait and limb ataxia, dysarthria, slow saccadic eye movements, supranuclear opthalmoplegia, peripheral neuropathy and parkinsonism. Expansion of an unstable CAG repeat in the exon1 of the SCA2 gene, located on chromosome 12q23-24.1, is responsible for this disease. Normal alleles showed a CAG repeat number ranged from 14 to 31 repeats frequently interrupted by one or more CAA trinucleotides, whereas the pathological expanded alleles contain a pure uninterrupted stretch of 33 to 200 repeats or more as reported in neonatal cases. We analyzed a SCA2 Italian family originates from an isolated village of middle-eastern Sicily. Following informed consent, genomic DNA was extracted from peripheral blood leukocyte samplings. SCA2 genotyping was performed by capillary electrophoresis on an ABI 310 Apparatus. Molecular analysis of SCA2 gene in our family revealed a patient carrying two expanded alleles with 35 and 36 uninterrupted CAG repeats. His brother and his nephew were heterozygotes for expanded allele with 35 and 42 CAG repeats respectively. We reported a patient homozygous for SCA2 disease mutation. This patient showed an earlier age at onset and a more severe clinical phenotype than his heterozygous brother who developed SCA2 thirty years later. The age at onset, the progression rate and severity of the SCA2 disease in the homozygous subject were similar to those observed in his heterozygous nephew carrying 42 repeats. These findings suggest a gene-dosage effect of SCA2 mutations on the age at onset of the disease. Similar results have been shown by other authors in the SCA6, SCA3 and DRPLA genes. Accumulation of homozygous SCA2 patients will help to explain the molecular mechanism of gene dosage on the age at onset of SCA2 disease.

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Unexplained CAG/CTG expansions in Portuguese and American patients with movement disorders. *A.I. Seixas[1,2], S.E. Holmes[1], P. Coutinho[3], A.E. Hillis[4], C.A. Ross[1,4], J. Sequeiros[2], I. Silveira[2], R.L. Margolis[1,4].* 1) Div of Neurobiology, Dept of Psychiatry, Johns Hopkins Univ School of Medicine, Baltimore, MD; 2) UnIGENe, IBMC, Univ of Porto, Portugal; 3) Serviço de Neurologia, Hospital São Sebastião, Feira, Portugal; 4) Dept of Neurology, Johns Hopkins Univ School of Medicine, Baltimore, MD.

Though great progress has been made in identifying the underlying genetic causes of hereditary movement disorders, the etiology remains unknown for as many as 30% of cases of dominant late-onset spinocerebellar ataxias, most cases of sporadic ataxia, and a smaller but not insignificant number of cases with Huntingtons disease-like syndromes. Twelve neurological disorders are caused by expansions of CAG/CTG repeats, and we hypothesize that additional CAG/CTG repeat expansion disorders exist, though these are likely to be rare. We used the RED (repeat expansion detection) assay to identify new pathogenic CAG/CTG repeat expansions in American and Portuguese patients with movement disorders of unknown cause. After RED analysis, DNA samples from individuals with CAG/CTG repeat length of > 40 triplets were tested for the presence of one of the known nonpathogenic repeat expansions. Among 67 patients from 43 Portuguese families with autosomal dominant ataxia and 95 patients with sporadic ataxia, we detected expansions in RED in 63 cases, all accounted for by nonpathogenic expansions at the *ERDA1* or *SEF2-1 loci*. In 138 patients from the US, at least two individuals have CAG/CTG expansions that cannot be explained by any known expansion. One patient presented with tremor, hyperreflexia, severe apraxia, a somewhat stiff gait, and cognitive impairment with prominent parietal deficits. The second patient has familial dementia, treatment resistant depression, and possible choreiform movements. These results suggest that additional CAG/CTG repeat expansion disorders remain to be described.

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Modifier genes are responsible for meconium ileus in cystic fibrosis but a major modifier gene is not located on chromosome 19q13. *R.S. Deering[1], S.M. Blackman[1], K. Naughton[1], R. McWilliams[1,2], B. Coleman[1], T. Lai[1], M. Algire[1], S. Beck[3], J. Hoover-Fong[1], A. Hamosh[1], M.D. Fallin[1], G.R. Cutting[1].* 1) Johns Hopkins University, Baltimore, MD; 2) Rutgers University and the UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 3) St. Christopher's Hospital for Children, Philadelphia, PA.

Meconium ileus (MI) is a potentially fatal intestinal obstruction that afflicts about 15% of newborns with cystic fibrosis (CF). Familial clustering suggests that shared genetic and/or shared environmental factors underlie MI. A role for genetic modifiers has been supported by linkage of MI to a region on chromosome 19 called CF Modifier gene 1 (CFM1). Using clinical data and DNA collected by the CF Twin and Sibling Study, we analyzed 62 monozygous (MZ) twin pairs, 20 dizygous (DZ) twin pairs, one set of DZ triplets and 250 sets of siblings to determine the role of genetic and other factors in MI and in distal intestinal obstruction syndrome (DIOS), a condition clinically similar to MI that occurs in older CF patients. Concordance for the presence of MI was highest in MZ twins (14 of 15 pairs, 93.3%) while concordance was significantly lower in DZ twins (2 of 9, 22%) and siblings (16 of 69 with 2 affected, 23% and 2 of 10 with 3 affected, 20%). Concordance rates for DIOS did not differ among MZ twins, DZ twins or siblings. However, DIOS was found to occur at significantly higher rates and was diagnosed significantly earlier in patients with MI treated by surgery than in patients with MI treated medically or those who did not have MI. One hundred and eighty-six families were typed with 7 DNA markers within the CFM1 region previously linked to MI. Nonparametric linkage analysis, multipoint parametric linkage analyses, single marker and haplotype transmission analyses did not support the existence of a major modifier gene on chromosome 19. Thus, intestinal obstruction in CF patients in the postnatal period is primarily due to non-genetic factors while intestinal obstruction in the neonatal period is due to genetic factors not located within CFM1 on chromosome 19.

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A new locus for autosomal recessive spastic paraplegia (SPG30) on chromosome 2. *S. Klebe[1], H. Azzedine[1], A. Durr[1,2], P. Bastien[3], N. Bouslam[1], N. Elleuch[1], S. Forlani[1], C. Charon[4], M. Koenig[5], J. Melki[6], A. Brice[1,2], G. Stevanin[1,2].* 1) IFR Neurosciences, INSERM U679-NEB, PARIS, France; 2) Dept Of Genetics, Salpetriere Hospital, APHP, Paris, France; 3) Gerardmer, France; 4) CNG, Evry, France; 5) IGBMC, Illkirch, CU Strasbourg, France; 6) INSERM E223, Evry-Val d'Essone University, Evry, France.

The hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by progressive spasticity in the lower limbs variably associated with neurological and extra-neurological signs. Approximately 30 different loci (SPG) have been mapped so far and 11 genes have been identified. While ~50% of dominant cases are explained by the known loci, less is known about autosomal recessive HSP (ARHSP) since the mapped loci have been identified in few families and account for only few percent of patients. We selected 10 ARHSP families (50 individuals, 20 of which where affected) without mutations in the SPG7 gene (Paraplegin). One large family of Algerian ancestry living in Eastern-France (7 unaffected and 4 affected members), was included in a genome-wide scan. Evidence of linkage was obtained at 2 consecutive markers on chromosome 2 with a multipoint lod score of 3.1. Six other possible locations (lod scores >1) were excluded using additional markers. Analysis of 9 additional microsatellite markers on chromosome 2 generated significant pairwise lod scores >3 with a multipoint lod score of 3.8 obtained in a 5 cM interval where all markers were homozygous in patients. Linkage to this new locus (SPG30) was excluded in 9 other ARHSP families. The phenotype of the SPG30 family consists in spastic paraparesis that started between 12 and 21 and was associated with distal wasting and saccadic ocular pursuit. In one case, electrophysiological examination showed the presence of a peripheral neuropathy. Cerebellar signs when present were very mild and limited to saccadic pursuit in 2 patients and difficult tandem standing in one.

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SPG29, a new locus for a pure form of autosomal recessive spastic paraplegia on chromosome 14q. *G. Stevanin[1,2], C. Paternotte[3], P. Coutinho[4], S. Klebe[1], N. Elleuch[1], E. Denis[1], A. Durr[1,2], J-F. Prudhomme[5], A. Brice[1,2], J. Hazan[3,6].*
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Hereditary spastic paraplegias (HSP) constitute a clinically and genetically heterogeneous group of disorders characterized by progressive spasticity in the lower limbs, due to the dysfunction and/or degeneration of the upper motor neurons. Molecular studies have confirmed and extended genetic heterogeneity and ~30 loci have been mapped to date. The pure forms of autosomal recessive HSP (ARHSP), in which pyramidal signs (brisk reflexes, Babinski sign, spasticity, motor deficit, +/- sphincter disturbances) are often associated with deep sensory loss without any additional symptoms, are less frequent than complicated ones and account only for 4 ARHSP loci: SPG5, 24, 27 and 28. We have identified a Portuguese consanguineous family with 3 affected sibs. Affected family members (1 man, 2 women) showed an early onset of pure spasticity within the first decade of life. After the exclusion of known ARHSP loci (namely SPG5, 7, 11, 15 and 28), a genome-wide study using 250 microsatellite markers has allowed the exclusion of more than 90% of the genome with the exception of a large homozygous region on chromosome 14. Linkage analysis using 49 microsatellite markers resulted in a maximal multipoint lod score of 3.13 with the ALLEGRO software whereas a multipoint lod score of 3.74 was reached with 12 markers using MAPMAKER/HOMOZ. Haplotype reconstruction restricted the candidate region to a 31-cM interval encompassing 29 homozygous markers in the 3 consanguineous patients. The critical interval is flanked by markers D14S264 and D14S58. Moreover, linkage to this newly identified locus, SPG29, has been excluded in 18 additional ARHSP pedigrees.

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X-Linked Optic Nerve Atrophy. *A. Azimi, L. Xue, J. Baird, E. Pearson, Y. Zhao, S. Kamaya, J. Meyer, Z. Yang, K. Zhang.* Ophthalmology, EIHG Room 2290, Salt lake city, UT.

Introduction: The primary inherited optic atrophy is a heterogeneous group of inherited disorders that result in loss of retinal ganglion cells, leading to the clinical appearance of optic atrophy. It affects between 1:10,000 to 1:50,000 people. The main clinical features are loss of visual acuity, color vision abnormalities, centro-caecal visual field defects, and optic nerve pallor. Optic atrophy may be sporadic or familial. The mode of inheritance may be autosomal dominant, autosomal recessive, X-linked recessive or mitochondrial. Within each of these groups, the phenotypic characteristics vary in such features as the age of onset, the severity of the visual loss, the color deficit and the overall prognosis. Methods: We conducted clinical and genetic investigation on a large family with x-linked optic atrophy. Individuals from a large family with x-linked optic atrophy underwent ophthalmologic and neurologic evaluations, and blood drawing. Six males in four generations were diagnosed with optic atrophy. Clinical features included onset of age in early childhood, decreased central vision and color vision, and optic nerve pallor. No abnormality was described in obligatory heterozygote carriers. Genotyping to X-linked optic atrophy (OPA2) were performed. Results: Genotyping to X-linked optic atrophy (OPA2) were performed. Positive linkage was obtained with STR markers linked to OPA2. Conclusion: We have identified a large family of x-linked optic atrophy. Genetic mapping localized the disease gene to OPA2. The identification of OPA2 gene will further our understanding of the pathophysiology of optic atrophy. It will also improve diagnosis, counselling and management of patients, and eventually lead to the development of new therapeutic modalities.

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Branchio-oto-renal syndrome: Seven novel Mutations in the EYA1 gene and possible genetic heterogeneity. *R. Uppala[1], C. Holley[1], F. Hildebrandt[2], CorW.R. Cremers[3], D.J. Orten[1], S. Kumar[1], W.J. Kimberling[1].* 1) Dept. Genetics, Boys Town Natl Research Hosp, Omaha, NE; 2) Dept. of Pediatrics & Communicable Diseases, University of Michigan, Ann Arbor. USA,; 3) Dept. of Otorhinolaryngology, University Hospital Nijmegen, Nijmegen, The Netherlands.

Branchio-oto-renal syndrome (BOR;MIM 113650) is an autosomal dominant disorder characterized by the association of bronchial cysts or fistulae, external ear malformations and/or preauricular pits, hearing loss and renal anomalies. It occurs at a frequency of approximately 1:40,000 live births. Mutations in the EYA1 gene at chromosome 8q13.3 and SIX1 at chromosome 14q23, are associated with BOR. A total of 41 loss-of-function mutations or missense mutations have been detected in EYA1. Recently, 3 different mutations in SIX1 were identified in BOR families (Proc Natl Acad Sci U S A 101:8090-5, 2004). Another locus on chromosome 1q31 was noted (Am J Hum Genet 66:1715-20, 2000), however the causative gene is yet to be identified. We analyzed the EYA1 and SIX1 gene coding regions from 87 independent BOR families/sporadic cases of various ethnic origins and identified seven novel EYA1 mutations in four independent families and 3 sporadic patients. Family SK-105 had a missense mutation, Y494C, in a highly conserved amino acid domain. A frameshift mutation due to 1-nt deletion was found in family SK-365, resulting in a truncated protein of 207 amino acids. Family SK-358 also had a missense mutation, R317S. Family SK-1396 had a frameshift deletion, resulting in a truncated protein of 521 amino acids. Family SK-2164 had a frameshift insertion, resulting in a truncated protein of 260 amino acids. Family SK-2193 had a nonsense mutation, R328X. In family SK-3385, we identified a novel heterozygous splice site mutation in exon 6 of EYA1 which deletes the T within the conserved donor site GT most likely causing loss of function and/or haplo-insufficiency. No pathogenic mutations were identified in the SIX1 gene-coding region in all patients tested. Absence of mutations in EYA1 and SIX1 and exclusion of large families to the 8q13.3, 14q23 and 1q31 loci by linkage analysis indicates genetic heterogeneity within BOR.

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Familial autoimmune myasthenia gravis - results of a genome-wide scan. *M.A. Knight, G. Landouré, A.A. Taye, K.H. Fischbeck.* Neurogenetics Branch, NINDS/NIH, Bethesda, MD.

Myasthenia gravis is usually a sporadic condition, but about 4% of myasthenic patients have a positive family history. We studied an Italian-American kindred with parental consanguinity and 5 of 10 siblings affected with autoimmune myasthenia. Affected patients in the family had positive anti-acetylcholine receptor antibodies and other manifestations of autoimmune disease. The age of onset ranged from 50 to 79 years. Previous studies in this family excluded the HLA, T-cell receptor and acetylcholine receptor subunit genes. The consanguinity suggests an autosomal recessive form of myasthenia gravis, although other forms of inheritance are possible. Based on the hypothesis that the mode of inheritance is autosomal recessive, we looked for shared homozygosity in a genome-wide scan with markers spaced at 2 cM intervals. A region of shared homozygosity was found at chromosome 13q13-21. This segment of chromosome 13 is of interest because it has been shown to be deleted in B cell leukemia. We are now mapping the locus more precisely, and candidate genes are being screened to identify the disease-causing mutation. We believe that this represents a new locus for autoimmune myasthenia gravis, and we are seeking additional families to confirm the localization.

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Mutation Hot-spot in CHX10 Gene Associated with Non-syndromic Microphthalmia in Qatari Families. *M. Ul Haque[1], S.H.E. Zaidi[2], M. Al-Mureikhi[3], G. Al-Thani[3], L-C. Tsui[4], A.S. Teebi[5].* 1) Molecular Genetics lab, DPLM, King Faisal Specialist Hospital, Riyadh, Saudi Arabia; 2) Division of Cardiology, Dept of Medicine, Univ Health Network, Toronto, ON, Canada; 3) Dept Pediatrics, Hamad Medical Corp, Doha, Qatar; 4) The University of Hong Kong, Pokfulam Road, Hong Kong; 5) Section of Clinical Genetics and Dysmorp, The Hosp for Sick Children, Toronto, ON, Canada.

Severe microphthalmia/clinical anophthalmia is a clinically heterogeneous disorder of eye formation, ranging from small size of a single eye to complete bilateral absence of ocular tissues. Null mutations in the CHX10 gene have been reported in non-syndromic microphthalmia in humans and in mice. CHX10 is expressed early in development in dividing retinal progenitor cells and antisense inhibition of Chx10 mRNA impaired retinal development in zebrafish embryos. We studied two unrelated highly consanguineous large families from Qatar in which affected individuals exhibited autosomal recessive form of non-syndromic microphthalmia. After informed consents were obtained, DNA samples from six affected and eight unaffected individuals from these families were subject to genetic analysis. Assuming a founder effect in each of the families, we performed homozygosity mapping using polymorphic markers adjacent to the CHX10 gene. In both families, linkage was evident for the CHX10 gene. Sequencing of the CHX10 gene of the affected individuals revealed a homozygous G to C substitution at nucleotide 599. The carrier parents were heterozygous for this mutation. This mutation resulted in replacement of the highly conserved arginine with proline (R200P). This R200P mutation has been previously reported in a mutation hot spot in micropthalmia patients from a Turkish family. Arginine 200 lies within the DNA recognition helix of the homeodomain of CHX10 and replacement of which results in severe disruption of the CHX10 binding to the DNA. Identification of the previously reported R200P mutation in ethnically different Qatari families, further confirms the functional importance of arginine 200 and suggests that this mutation may have a wide-distribution among different populations.

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Novel HSF4 mutation causes autosomal dominant congenital total white cataract in a Chinese family. *T. Ke*[1], *M.G. Liu*[1], *B.C Ji*[2], *Q. Wang*[1]. 1) Human Genome Research center,Huazhong University of Science and Technology, Wuhan, Hubei, 430074, China; 2) General Hospital of Jinzhushan Industrial Group Corporation, Hunan, 417505, china.

Congenital cataract is one of the most common abnormalities of the eye and a frequent cause of blindness in infants with a prevalence of 1-6 cases per 10,000 births. We report here a Chinese family with autosomal dominant congenital cataract. The cataract in the family appears to be the type of total white. Linkage and haplotype analyses mapped the disease-causing gene in the family to chromosome 16q22, and defined the disease gene to a 24.48-CM region. The disease interval contains the HSF4 gene encoding one member of the family of heat shock transcription factors that regulate the expression of heat shock proteins (Hsps) in response to different stresses. Direct DNA sequence analysis of all exons and exon-intron boundaries of HSF4 identified a nucleotide change c.221G>A in exon 2, which results in substitution of a highly conserved arginine residue by histidine (p.Arg74His) in the DNA-binding domain. The mutation cosegregated with all affected individuals in the family, but not in unaffected family members and more than 150 controls. These results identified a novel missense mutation (p.Arg74His) in the transcript factor gene HSF4 that causes total white cataract in a Chinese family, and provide the first independent confirmation that mutations in HSF4 can cause autosomal dominant congenital cataract.

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Novel mutation of the OPA1 gene causes autosomal dominant optic atrophy. *J. Liu[1], T. Ke[1], L. Zhou[1], M. Liu[1], Q. Wang[1,2].* 1) Huazhong University of Science and Technology Human Genome Research Center; 2) Center for Molecular Genetics, Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation.

Autosomal dominant optic atrophy (ADOA) is a progressive eye disorder caused by degeneration of the retinal ganglion cell layer with ascending atrophy of the optic nerve. The onset of ADOA is often within the first decade of life. The prevalence rate of ADOA is 1 in 50,000 individuals. Three different ADOA genes (OPA1, OPA3 and OPA4) are located on chromosomes 3q28-29, 19q13.2-13.3 and 18q12.2-12.3, and OPA1 and OPA3 have been identified. In this study, we studied a 9-member, three-generation Chinese family with ADOA. The proband and one of her two affected sons displayed mental retardation, and the other affected son has short stature. We selected markers located at the three regions, and performed linkage analysis. Linkage was identified with two markers that span the OPA1 gene, D3S1601 and D3S1311. The OPA1 gene encodes a 960 amino acid protein with homology to dynamin-related GTPases. The OPA1 gene comprises of 28 exons and spans more than 40 kb of genomic sequence. Direct DNA sequence analysis of all exons and exon-intron boundaries revealed a novel mutation in exon 12, a G-to-A transition at nucleotide 1,202 of the OPA1 gene, resulting in a Gly401Asp substitution in the GTPase domain (encompassing the core central region between amino acid residues 280 and 520). RFLP analysis showed that the Gly401Asp mutation co-segregated with all patients in the family, and did not exist in unaffected family members and 200 unrelated normal controls. The OPA1 gene is widely expressed, but is most abundant in the retina. The Gly401Asp mutation may disrupted the GTPase activation of OPA1 in the retina and causes autosomal dominant optic atrophy in the family. Our results identify a novel mutation in OPA1 gene that causes optic atrophy associated with mental retardation and short stature.

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Identification of a novel splice site mutation in collagen type VII gene that causes autosomal dominant dystrophic epidermolysis bullosa pruriginosa in a Chinese family. *X. Ren[1], M. Liu[1], J. Liu[1], Q. Yao[1], L. Zhai[2], Q. Wang[1].* 1) Huazhong University of Science and Technology Human Genome Research Center; 2) Nanyan the First People's Hospital.

The dystrophic form of epidermolysis bullosa pruriginosa (DEB) is characterized by extensive scarring due to sublamina densa blistering of the skin. Both autosomal dominant and autosomal recessive forms of DEB have been reported and are caused by different mutations in the type VII collagen gene (COL7A1). We report here a Chinese family with autosomal dominant DEB. Linkage analysis suggests that the family is linked to COL7A1. DNA sequence analysis and single stranded conformation polymorphism identified a substitution at the 87th exon/intron boundary (IVS87 +1 G -> T) in all affected family members, but not in the unaffected family members and 200 normal controls. Reverse-transcription PCR with total RNA from skin biopsies from the proband revealed that IVS87 +1 G -> T causes the deletion of exon 87. The mutant protein encoded by the truncated transcript is expected to lack a part of the triple helical domain involved in anchor fiber assembling. These results identify a novel splicing mutation in COL7A1 that causes DEB in a Chinese family.

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Molecular Characterization of a UK Family with Posterior Polymorphous Corneal Dystrophy. *C. Mok[1], W. Ferrini[1,2], C. Willoughby[3], M.A. Walter[4], Y. He[5], E. Héon[1,2].* 1) Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Ophthamology and Vision Science, Queen's University Belfast, Royal Victoria Hospital, Belfast, Northern Ireland; 4) Chair and Associate professor, Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 5) Cytogenomics Facility, The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada.

We investigated a four-generation family for the genetic causes of posterior polymorphous corneal dystrophy (PPCD) and keratoconus (KC). The family exhibited an autosomal dominant pattern of inheritance along with full penetrance. One affected patient showed some features of rieger malformation. Genotype analysis, using STRP markers, was performed on seven candidate loci for PPCD, KC and Axenfeld-Rieger syndrome. Candidate genes were analysed using direct cycle sequencing. Linkage and haplotype analysis excluded all but one of the candidate loci. Suggestion of linkage was observed for the FOXC1 locus (6p25). Gene dosage and cytogenetic analyses were performed to assess the role of chromosomal rearrangement, gene deletion or duplication. No disease-associated changes or microdeletions/duplications were observed within the FOXC1 locus. No disease-associated coding sequence changes were observed in FOXC1, FOXF2, FOXQ1 and a highly conserved CpG island upstream of FOXC1. Cytogenetic analysis of an affected patient resulted in a normal karyotype while FISH analysis of the 6p25 locus revealed no microdeletions or translocations. Signals were seen at chromosome 16p11.2 (RP11-1083C15) and 11q2 (RP11-1104N17) in both our affected patient and controls. To our knowledge the large-scale polymorphism on chromosome 11 has not been previously described. The disease-associated change does not appear to involve the 6p25 FOX genes family.

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Splicing of exon 5 of the MCAD gene and exon 7 of the SMN1/2 genes is dependent on exon splicing enhancers that counteract an identical exon splicing silencer - An example of a common architecture for splicing regulatory elements in two genes. *B.S. Andresen[1,2], K.B. Nielsen[1,2], P.P. Madsen[1,2], S. Sorensen[3], T.K. Doktor[1,2], L. Cartegni[4,5], A.R. Krainer[4], T.J. Corydon[1], J. Kjems[3], N. Gregersen[2].* 1) Inst Hum Genet, Aarhus Univ, DK; 2) Res Unit f Molec Medicine, Clin Inst, Aarhus Univ and Skejby Sygehus, DK; 3) Dept of Molec Biol, Aarhus Univ, DK; 4) Cold Spring Harbor Lab, New York, USA; 5) Sloan-Kettering Cancer Center, New York, USA.

Exon skipping caused by exonic point mutations is an intriguing molecular defect mechanism that is not fully understood even in the best studied examples. In spinal muscular atrophy (SMA) the SMN 2 gene can not compensate for the loss of the SMN1 gene because an exonic C>T mutation causes exon 7 skipping. Different models try to explain this: 1. Inactivation of an SF2/ASF binding exon splicing enhancer (ESE); 2. Creation of an hnRNPA1 binding exon splicing silencer (ESS). We have identified a mutation (362C>T) in exon 5 of the MCAD gene that causes exon skipping in patients. Mutagenesis studies in MCAD minigenes showed that 362C>T inactivates an ESE that is similar to the exon 7 SMN1 ESE. Both ESEs functions in a heterologous context. Moreover, the MCAD ESE could be substituted with the SMN1 ESE, whereas the corresponding SMN2 sequence caused exon 5 skipping. Optimization of the acceptor splice site of MCAD exon 5 normalized splicing from all constructs. Surprisingly, introduction of a flanking polymorphic variation (351A>C) corrected splicing from all mutant MCAD minigenes (Incl. those with the SMN2 seq.). Mutagenesis showed that 351A is part of an ESS, which is identical in the MCAD and SMN genes, and identical to an hnRNPA1 binding ESS in HPV-16 L1. In vitro RNA experiments showed that hnRNPA1 binding was determined by the combined activity of the ESE and the ESS. We suggest a common model for splicing regulation of SMN exon 7 and MCAD exon 5, in which the MCAD 362C>T and the SMN2 C>T mutations inactivates an ESE that is needed to prevent (hnRNPA1?) binding to the flanking ESS. Binding to the ESS inhibits recognition of the weak acceptor splice site and causes exon skipping.

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Canadian CF Modifier Gene Project: candidate gene analysis. *J. Zielenski[1], R. Dorfman[1], A. Sandford[2], M. Corey[1], D. Markiewicz[1], X.-W. Yuan[1], A. Master[1], I. Evtoushenko[1], Y. Sang[1], F. Lin[1], V. Wang[1], C. Taylor[1], P. Pare[2], L.-C. Tsui[3], P. Durie[1], and Canadian Cystic Fibrosis Clinics.* 1) Hospital for Sick Children, Toronto, ON, Canada; 2) iCAPTURE/University of British Columbia, BC, Vancouver, Canada; 3) University of Hong Kong, Hong Kong, China.

Severity of clinical phenotype of cystic fibrosis (CF) is determined both by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene as well as variations in other genes (CF modifiers). One of the approaches to identifying CF modifiers is analysis of candidate genes selected on the basis of their relevance to various aspects of CF disease. The Canadian CF Modifier Gene Project is a family based approach to identifying and characterizing genes that modulate clinical severity of cystic fibrosis. The Project involves: (1) blood sample and data collection from CF families and (2) analysis of association of CF modifier loci and genes with various clinical variables in CF patients. To date, 38 Canadian CF Clinics and 7 foreign CF centers participate in the Project. Study consents were obtained from 2039 CF families. Currently, 10020 blood samples were obtained from 4939 individuals (patients and parents) for genomic DNA extraction and lymphoblast cell line establishment (patients only). Demographic, cross-sectional and longitudinal data are being collected from medical charts of participating CF patients. Approximately 65% of the data have been collected from charts of participating patients and reviewed for quality. In our candidate gene approach, 79 genes with relevance to CF have been selected as potential modifiers of clinical severity in CF patients. Genotyping of 161 intragenic markers is at various stages of progress. These include markers from genes previously implicated as CF modifiers. Our preliminary results using Family-Based Association Tests (FBAT) and/or Analysis of Variance (ANOVA) confirm the previously reported association of two of these genes: transforming growth factor beta 1 (TGFbeta1, G74C/R25P) and tumor necrosis factor alpha (TNFalpha, -A308G) with CF patients pulmonary function. (Supported by Genome Canada through Ontario Genomics Institute and Canadian CF Foundation).

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DNA analysis in differential diagnosis of hepatic bilirubin metabolism disorders. *A. Krumina[1], I. Micule[1], V. Sondore[2], J. Keiss[1, 2].* 1) Dept. of Medical Biology $ Genetics, Riga Stradins University, Riga, Latvia; 2) Infectology Center of Latvia, Riga, Latvia.

The most common disorder of hepatic bilirubin metabolism in humans is Gilbert syndrome (GS). Traditionally, the diagnosis of GS has been a diagnosis of exclusion, made when mild unconjugated hyperbilirubinaemia has been observed in a patient with no evidence of haemolysis and in whom liver function tests are within normal values. Diagnostic criteria for GS in Latvia includes mild elevation of serum bilirubin, which is at least 90 percent unconjugated, and decreased level of glucuronides in daily urine. A new tool for the definitive diagnosis of GS has been provided by finding TA insertion in the promoter of the gene UGT1A1. Gene UGT1A1 encodes for the enzyme uridine diphosphate-glucuronosyltransferase, which conjugates bilirubin with glucuronic acid. TA insertion in the promoter region of the gene UGT1A1 is observed in nearly all the patients with GS and in 12 - 15% of healthy individuals. The **aim** of the study was to estimate the role of DNA analysis in the diagnosis of GS. **Material** was DNA isolated from the peripheral venous blood. **Objects** were 72 patients with a clinical diagnosis of GS. **Method** used was denaturing polyacrylamide gel electrophoresis of the amplified promoter region of the gene UGT1A1. **Results**. The following variants of TA dinucleotide polymorphisms in the promoter region of the gene UGT1A1 were observed: $(TA)_7/(TA)_7$ - in 69 individuals (95.8%), $(TA)_6/(TA)_7$ - in 2 (2.8%), $(TA)_6/(TA)_6$ - in 1 (1.4%). Elevated level of unconjugated hyperbilirubinaemia and decreased level of glucuronides in daily urine were found in 86.6% and 88.8% patients, respectively. **Conclusion**: The necessity to improve differential diagnosis of GS among all the other cases of unconjugated hyperbilirubinaemia requires the inclusion of DNA analysis in the diagnostic algorithm of Gilbert syndrome.

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Embryonic lethality in mice deficient in *tex292*, ortholog of the North American Indian childhood cirrhosis (NAIC) gene *CIRH1A*. *B. Yu, G.A. Mitchell, A. Richter.* Medical Genetics, Sainte-Justine Hospital, Montreal, Canada.

NAIC is a severe progressive nonsyndromic autosomal recessive intrahepatic cholestasis. *CIRH1A*, mapped to 16q22, encodes cirhin, a 686 residue nucleolar protein with an active C-terminal monopartite nuclear localization signal and a unique nucleolar localization signal. All known NAIC patients are homozygous for the exon 15 mutation R565W, which alters the predicted secondary structure of cirhin but produces a stable, accurately-targeted protein. *Tex292* is widely expressed, particularly in liver, both postnatally and in 11.5 days pc mouse embryos. We studied mice generated with the gene trap vector pGT1Lxf, produced from Bay Genomics ES cell line XH230. This vector has a splice acceptor upstream of the -geo reporter gene, a fusion of -galactosidase and neomycin phosphotransferase genes (Stryke et al, NAR 31: 278, 2003). We precisely characterized the insertion in XH230 and found integration between exons 9 and 10 in *tex292*. Two of 3 chimeras produced germ-line transmission. In heterozygote crosses with average litter size of 5.3 we found a ratio of offspring genotypes suggestive of prenatal lethality: normal, 36; heterozygous, 59; homozygous mutant, 0. The oldest heterozygotes are now 24 weeks old and appear normal for growth and activity; evaluation of their liver function and histology is in progress. To investigate the timing of the embryonic lethality, we proceeded to examine embryos at 11.5, 9.5 and 8.5 dpc. A total of 14 gravid females were sacrificed. The genotyping of 81 embryos failed to reveal homozygous targeted embryos while the expected ratios of normals and heterozygotes were detected. Staining of the embryos for -galactosidase confirmed the genotyping. We are now studying earlier embryos. In mice, complete *tex292* deficiency is incompatible with survival past 8.5 dpc, and perhaps earlier. The distinct, liver-specific phenotype of NAIC patients may reflect species specific influences or may be related to the specific properties of the R565W mutation.

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MDR3 (ABCB4) mutations are not an uncommon cause of contraceptive pill-induced cholestasis and familial cholestasis. *M. Hrebicek[1], M. Bouckova[1], L. Dvorakova[1], J. Horak[2], J. Sperl[3], P. Trunecka[3], M. Jirsa[3].* 1) Institute of Inherited Metabolic Disorders, Prague, Czech Republic; 2) 2nd Department of Internal Medicine, 3rd Faculty of Medicine, Prague, Czech Republic; 3) Institute of Clinical and Experimental Medicine, Prague, Czech Republic.

The ABCB4 (MDR3) is a phospholipid pump located predominantly at the biliary pole of the hepatocyte. Mutations in the *ABCB4* gene were found in homozygosity or heterozygosity in patients with progressive familiar intrahepatic cholestasis type 3 (PFIC3), cholesterol lithiasis (CL) and intrahepatic cholestatis of pregnancy (ICP). The *ABCB4* gene is located at chromosome 7q21.11 and spans more than 75 kb. We have analysed 27 protein-encoding exons of *ABCB4* gene in 6 Czech families with cholestatic disease, with familiar occurence of cholesterol lithiasis and contraceptive pill induced cholestasis. In three of the families the probands carried on one of the alleles a mutation - c.3608C>G (S1203X), c.1501G>T (E501X), and c.1954A>G (R652G), respectively, and wild type sequence on the other. The fourth proband was a compound heterozygote for mutations c.523A>G (T175A) a c.1371del G (G457X). In two of the families no apparently pathogenic mutations were found. The fifth proband was heterozygous for 3 synonymous mutations (c.175C>T, L59L; c.504T>C, N168N; c.711A>T, I237I) and the sixth proband carried on one of the alleles the IVS28-16C>T that is likely not pathogenic. These findings confirm that mutation analysis of *ABCB4* should be considered in the broadening spectrum of hepatic diseases including cholestasis induced by hormonal contraceptives and familial cholelithiasis with onset before 25 years of age. Treatment with ursodeoxycholic acid has a potential to ameliorate the symptoms of ABCB4 deficiency and the patients may profit from early diagnosis. Supported by grant IGA MZ CR NM/7510-3.

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Human Inherited Disorders with CNS Phenotype: Types of Genes. *G. Bruns*[1,2]. 1) Genetics Div, Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Medical School, Boston, MA.

As part of a study of the nature of genes underlying different types of developmental disorders, the OMIM Morbid Map was sequentially searched for inherited diseases with a significant central nervous system component and known gene mutation. In many of these syndromes, other organ systems were also involved. Disorders with transient neurologic symptoms secondary to acute metabolic decompensation were excluded. When mutation in a gene can lead to more than one syndrome with a CNS phenotype, that gene was tabulated only once. Of the ~300 inherited diseases with known mutations evaluated to date, twenty-two percent (66 disorders) included a CNS phenotype. Among these, mutation of genes specifying biosynthetic metabolic pathway enzymes was the most common - 13 disorders (20 percent). Seven mutations disrupted lipid or lipoprotein catabolism (10.6 percent). Seven involved fundamental neural cell proteins or neural cell growth factors; 6 mutations impacted excision repair DNA binding proteins and DNA helicases; 6 involved signal transduction or cell fate molecules; and 3 disrupted zinc finger or paired box transcription factors. Five mutations affected organelle trafficking proteins, specifically peroxisomal translocases. Two disorders derive from mutation of genes for metal binding/transporting proteins and 2 from alteration of solute carrier family members. In 12 of the disorders with a CNS phenotype (18.2 percent), the mutation(s) involved a novel protein, nearly all with extensive homology in lower Eukaryotes. A number of these have recognizable domains/motifs including TM domains. Although the frequencies and categories of mutations reflect the disorders evaluated to date, alteration in metabolic pathway biosynthetic enzymes, lipid/lipoprotein catabolism, fundamental neural cell proteins, DNA repair factors, signal transduction molecules, and novel proteins of ancient origin appear to be prominent in inherited diseases that involve the central nervous system. Mutations in genes that specify proteins restricted to higher Eukaryotes were infrequent.

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Screening for haploinsufficiency of *TCOF1* mRNA in Treacher Collins syndrome patients. *K.H. Shows, R. Shiang.*
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The majority of mutations in *TCOF1* identified to cause Treacher Collins syndrome (TCS) are nonsense, deletion, and insertion mutations that lead to a premature stop codon and can cause truncation of the expressed protein treacle or lead to nonsense-mediated mRNA decay. It has been proposed that the mechanism of action of a heterozygous nonsense mutation is through haploinsufficiency of functional treacle during a critical time point in neural crest cell development, resulting in the craniofacial abnormalities characteristic of TCS. We performed quantitative real time PCR using RNA derived from lymphoblastoid cells to investigate the occurrence of haploinsufficiency of *TCOF1* mRNA in TCS patients with known mutations in *TCOF1*, in TCS patients who showed no mutations after *TCOF1* screening, and in normal individuals. Results show that there is no pattern of association of mRNA level with presence or absence of known or unknown *TCOF1* mutation; therefore, screening for haploinsufficiency of *TCOF1* mRNA in lymphoblastoid-derived RNA is uninformative in our sample.

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Study of C2orf34, a novel gene not expressed in the 2p16 deletion syndrome. *S. Buriakovsky, M. Rubinshtein, R. Parvari.* Developmental Molecular Genetics, Ben Gurion University, Beer Sheva, Israel.

We identified Bedouin patients presenting a unique recessively inherited syndrome, with broad clinical manifestations: cystinuria, mental & growth retardation, hypotonia, facial dysmorphism and mitochondrial respiratory chain dysfunction presenting as reduced activity of all mitochondrial encoded respiratory chain enzymatic complexes. Our previous study identified the molecular basis of this syndrome as a homozygous deletion of 179,311 bp on chromosome 2p16, and thus was subsequently named the 2p16 deletion syndrome. Further studies to define the transcription content of this interval showed that it includes 4 protein coding genes: type I cystinuria SLC3A1, protein phosphatase 2C, and two uncharacterized genes PREPL and C2orf34 . In addition, the presence of three transcripts without, or with very short, open reading frames was noted in the deleted region. The C2orf34 gene has its promoter and first exon in the deletion, verification by RT-PCR using patients' RNA demonstrated that it is not expressed in patients lymphoblastoid and fibroblasts cells in contrast to RNA from cells of controls. Bioinformatics investigation suggests that one of the two splice variants of the gene, not expressed in the 2p16 deletion syndrome patients, may have methyltranferase activity and is highly conserved during evolution. RT-PCR using RNA of various tissues demonstrates that the two spliced forms are ubiquitously expressed, including in the tissues affected by the syndrome. GFP tagging suggests different sub cellular localizations of the spliced forms one to the nucleus and the other to the Golgi compartments. We hypothesize that the absence of this gene in the patients may have a major contribution to the clinical presentation of the patients.

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Tissue Polyamines and Polyamine Regulation in Arginase Knockout Mouse Models. *J.C. Livesay[1], J.L. Deignan[1], P.K. Yoo[1], A.E. Pegg[2], R.K. Iyer[1], S.D. Cederbaum[1], W.W. Grody[1].* 1) Departments of Pathology and Laboratory Medicine, Psychiatry, Pediatrics and Human Genetics and The Mental Retardation Research Center, David Geffen School of Medicine at the University of California, Los Angeles, CA; 2) Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA.

Defining specific and distinct roles for arginases AI and AII has been a complex task. AI was thought to be primarily involved in ureagenesis while AII was thought to be involved in the production of ornithine for all other functions such as the biosynthesis of polyamines and as a part of the inflammatory process. Recent in situ hybridization and RT-PCR studies in our lab have shown that AI is actually more widely expressed than AII in adult animals, contradicting these former assumptions. A plausible new role for AI outside of the liver is the production of ornithine for the biosynthesis of polyamines, which are essential for cell proliferation and differentiation. To test this hypothesis, we previously created an AI knockout mouse, and after acquiring the AII knockout mouse, created the double knockout in order to look for any alterations in tissue polyamines in the absence of arginase. Analysis of polyamines in the liver, brain, kidney, and small intestine of arginase single and double knockout mice revealed a lack of alterations in polyamines in all of these tissues. Subsequent RT-PCR analysis revealed that some of the other arginine metabolic enzymes were slightly altered in specific tissues; however, none of the polyamine regulatory enzymes analyzed showed any dramatic alterations in their expression in the various arginase deficient mice. These data reveal that, at least in the tissues analyzed, AI and AII do not serve a primary role in the production of ornithine for polyamine biosynthesis nor does the absence of either isoform alter the regulation of the polyamine synthetic enzymes in any discernible way. Future studies will determine if alterations in polyamines exist in male and female reproductive tissues in the arginase knockout mice, where arginase expression is likely to be more important for polyamine production.

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Is the IP male-phenotype caused by alteration of NEMO protein as in IP -female? *M. Ursini, F. Fusco, G. Fimiani, A. Pescatore, M.G. Miano, M. D'Urso.* Human Genetics Laboratory, IGB/ABT/CNR, Naples, Italy.

Incontinentia pigmenti, or Block-Sulzberger Syndrome, is an X-linked genodermatosis with heterogeneous phenotype. IP, classically considered a male-lethal disorder, results from mutations in the gene for NF-kB essential modulator (NEMO), with deletion of exons 4-10 of NEMO accounting for 180% of new mutations. Loss of function mutations of NEMO gene are lethal in male during embriogenesis while the female survive for an extremely skewed X-inactivation pattern. Less deleterious mutations that preserve the NEMO activity can result in survival of male subjects, but with ectodermal dysplasia and immunodeficiency (EDA-ID). We investigated the NEMO coding region in 18 male patients with clinical hallmarks of IP. Characteristic abnormalities of the skin and alterations of hair, nails, teeth, eyes and central nervous system were present, and EDA-ID phenotype was clinically excluded. We amplified and sequenced the coding region of NEMO from genomic DNA by PCR and we found the genomic deletion in 4 IP male: 3 resulted to be mosaic for the common mutation while 1 had a 47,XXY karyotype. The NEMO protein presence and the normal activation of NF-kB pathway after TNFalfa stimulation was demonstrated in skin fibroblast culture from two male patients carrying wild-type NEMO coding region. In 14 IP male patients that showed wild type NEMO coding gene, we carried out mutational search in the NEMO conserved non coding regions and in its 5-UTR using PCR amplification and direct sequencing methods. To date, 6 point mutations were identified, 4 in the 5UTR and 2 around the transcription start site and are currently under analysis. NEMO transcription regulation underlining IP pathogenesis and/or if other genes are involved in the IP-male phenotype remains to be evaluated.

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Detection of muscular dystrophy genotypes via universal condition direct sequencing (UCDS). *R.R. Bennett[1], H.E. Schneider[1], A. Lakdawalla[2], P.S. Lai[3], C.E. Barrett[1], V. Lip[1], B.L. Wu[1], B.T. Darras[1], L.M. Kunkel[1].* 1) Department of Genetics, Children's Hosp, Boston, MA; 2) Applied Biosystems, Foster City, CA; 3) Department of Pediatrics, National University of Singapore, Singapore.

Currently, mutations in many of the genes causing the various forms of muscular dystrophy remain undetected in patients because of the unavailability of a reliable and cost effective assay to detect them. One promising strategy requires the amplification of all salient portions of a gene or many genes at one single set of PCR conditions. This is achieved by designing amplicons of uniform large (~1000bp) size. Internal primers are then used for sequencing (Flanigan et al. 2003). A second strategy which we are developing in collaboration with Applied Biosystems, Foster City Ca. using its VariantSEQr resequencing system is to use automated primer design tools to seek primers for all salient portions of many genes. Using Amplitaq GoldPCR system, these amplicons will all amplify at a single set of PCR conditions. In addition, by using M13F and M13R tails on the primers, a universal sequencing plate can be utilized in this process.We have developed a high throughput 384 well plate robotic process with dried primers using the Applied Biosystems VariantSEQr resequencing system for genes associated with any form of muscular dystrophy. The new sequencing strategy provides more than an order of magnitude reduction in cost, time to detect mutation and complexity of analysis. Detection of mutations is facilitated rapidly using SeqScape v2.1 software (Applied Biosystems, Foster City Ca.). This software incorporates a consensus sequence and a tab process for tabing through all variations between patients and consensus, known polymorphisms versus unknown variants as well as insertions and deletions. As of June 2005, we have all assays working for this process called UCDS (Universal Condition Direct Sequencing) for the DMD, CAV3, TRIM32, CAPN3, FKRP, FCMD, DYSF, SGCA, SGCB, SGCD, SGCG, NEB genes using control DNA. Over the summer, we will be finalizing and publishing this process and continuing next year to develop new assays until all muscular dystrophy and myopathy genes are included.

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Sense from nonsense for primary pulmonary hypertension: screening transcripts protected from nonsense mediated decay enhances detection of BMPR2 mutations. *J.A. Phillips III[1], J.D. Cogan[1], L.K. Hedges[1], L.A. Wheeler[1], E.P. Dawson[2], J.E. Loyd[1].* 1) Depts of Pediatrics and Medicine, Vanderbilt University School of Medicine, Nashville, TN; 2) Bioventures Inc, Murfreesboro, TN.

Background: About 50% of cases with familial primary pulmonary hypertension (FPPH) are reported to have mutations in the bone morphogenic protein receptor type 2 (BMPR2) gene. BMPR2 has 13 exons, spans >180 kbs, and is 98% intronic. Since most reported BMPR2 mutations were found by PCR amplification and sequencing of exons and intron boundaries, they reside in exons or splice junctions. **Hypothesis:** Since exonic sequencing can miss duplications, deletions and inversions that cause Nonsense Mediated Decay (NMD), analysis of transcripts protected from NMD should be more efficient and sensitive in detecting BMPR2 mutations. **Methods:** Lymphoblastoid cells from 10 unrelated FPPH patients previously found to not have BMPR2 mutations by exon sequencing were incubated with and without puromycin. RNA was then isolated and reverse-transcriptase PCR (RT-PCR) products containing BMPR2 transcripts were sequenced. **Results:** Analysis of RT-PCR products from lymphoblastoid cells from 6/10 samples showed exon deletions or duplications, and BMPR2 transcripts from 1/2 of these were affected by NMD. Incubation of all cell lines having BMPR2 transcripts degraded by NMD with puromycin, which inhibits NMD, roughly equalized the proportions of mutant and normal BMPR2 transcripts. Of the remaining 4/10 samples without exon duplications or deletions, 3/4 (75%) were homozygous for all exonic SNPs. **Conclusions:** 1) BMPR2 mutations were found outside exons in 60% of unrelated FPPH samples in which exon sequencing was negative and RT-PCR products of 75% of the remaining samples were homozygous for exonic SNPs, 2) transcript analysis is a more efficient and sensitive method to detect BMPR2 mutations than sequencing amplicons containing exons, 3) puromycin can block NMD of BMPR2 transcripts in lymphoblastoid cells and 4) transcripts associated with duplications, deletions, inversions, stop codons, altered splicing and possibly promoter changes (by allelic dropout) can be efficiently detected using this method.

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Munc13-4 mutations in North American families with Hemophagocytic Lymphohistiocytosis. *K. Zhang[1], J. Biroschak[1], S. Lee[2], J. Villanueva[2], J. Johnson[1], AH. Filipovich[2], RJ. Wenstrup[1].* 1) Division of Human Genetics, Cinncinnati Children's Hosp, Cincinnati, OH; 2) Division of Hematology /Oncology, Childrens Hospital Medical Center, Cincinnati, Ohio, USA.

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder of immune regulation which typically presents in early childhood and has a rapidly fatal course unless aggressively treated with chemotherapy, immune suppression and bone marrow transplant. While earlier reports have identified mutations in the perforin (PRF1) genes in 20-30% of families with HLH, more recently, mutations in Munc13-4 have been observed to be responsible for about 10-30 percent of FHL cases in European and Japanese population. Munc13-4, a member of the human Munc13 family contains 32 exons that encodes a 123-kDa protein, which is involved in vesicle trafficking and membrane fusion. Patients with Munc13-4 mutations have impaired lymphocyte cytotoxic function with normal perforin expression. In this report we describe results of Munc13-4 gene mutation analysis in twenty-five unrelated North American families with children diagnosed with HLH without PRF1 mutations. Mutations of Munc13-4, including nine novel mutations and more than thirty sequence variants were identified in 22 families. Six probands carry bi-allelic pathogenic mutations in the Munc13-4 gene. Eight of ten nonsynonymous sequence variants were not found in the 50 normal individuals that from southern Ohio populations. Varying degrees of deficiency in natural killer (NK) cell function have been detected in this group of patients, indicating that Munc13-4 genotype-phenotype correlations are yet to be established. Interestingly, one patient with classical Macrophage Activation Syndrome and systemic onset Juvenile Rheumatoid Arthritis, who developed HLH-like syndrome, carried bi-allelic mutations in the Munc13-4 genes. Since approximately 25% of cases of HLH in North America are caused by Munc13-4 mutations, DNA-based screening for HLH due to Munc13-4 mutations using DNA sequencing is recommended in affected families when the PRF1 gene found to be normal.

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Spastin mutations in patients with sporadic spastic paraparesis. *C. Depienne[1,2], C. Tallaksen[1], J.Y. Lephay[1], B. Bricka[2], S. Poea-Guyon[1], B. Fontaine[3], P. Labauge[4], A. Brice[1,2], A. Durr[1,2].* 1) INSERM U679, Paris, France; 2) Unité de Neurogenetique, Departement de Genetique, Cytogenetique et Embryologie, AP-HP, Groupe Hospitalier Pitie-Salpetrière, Paris, France; 3) Federation de Neurologie and INSERM U546, Groupe Hospitalier Pitie-Salpetriere, Paris, France; 4) Service de Neurologie, CHU Montpellier-Nimes, Hopital Caremeau, Nimes, France.

SPG4, encoding spastin, is the major gene responsible for autosomal dominant hereditary spastic paraparesis (HSP), accounting for 10-40% of families with pure or complicated phenotype. The goal of this study was to assess the frequency of SPG4 mutation in a large series of patients with spastic paraplegia without family histories. We selected 147 mostly European probands with progressive spastic paraplegia of unknown origin and without transmission of the disease. DNA was screened by DHPLC for sequence variants in the 17 coding exons of the SPG4 gene and sequence variants were characterized by direct sequencing. We identified 19 different mutations, 13 of which were novel, in 18 different patients, for an overall mutation rate of 12%. The sporadic nature of the disease resulted from true reduced penetrance in one family in which both parents were found normal. There was no evidence for de novo mutation. The mutations associated with sporadic patients were mostly of the missense type and were sometimes located out of the AAA domain. We showed that the spectrum of mutations in sporadic patients is different from that of familial cases and is associated with a milder SP phenotype and a reduced penetrance. The high frequency of SPG4 gene mutations in patients with sporadic spastic paraplegia argues in favour of gene testing in patients with pure or complicated spastic paraplegia even without family histories, because it permits appropriate genetic counselling.

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Haplotype analysis in Indian Wilson disease patients. *G. Kaur[1], S. Kumar[2], B.R. Thapa[3], R. Prasad[2].* 1) Department of Physiology, Chandigarh Medical College, Chandigarh, India; 2) Department of Biochemistry, PGIMER, Chandigarh, India; 3) Department of Pediatric Gastroenterology, PGIMER, Chandigarh, India.

Wilson disease (WD) is a disorder of copper transport due to mutations in ATP7B gene. Till date, mutation screening in Indian WD patients has led to the identification of 22 mutations (1). Till now date, no data is available on alleles distribution and haplotype association with specific mutation in Indian WD population. Henceforth, in the present study, we have determined the alleles distribution and haplotype association with specific mutation by using D13S314, D13S301 and D13S316 dinucleotide markers in 28 WD patients. All three markers were found to be equally distributed. Total of 27 different haplotypes associated with 56 WD chromosomes were determined. Haplotypes 1-7-4, 5-3-2, 5-4-6, 6-8-7, 10-6-5 and 11-8-2 were common and being observed in 35% of WD chromosomes. Haplotypes 1-7-4 and 5-3-2 were most common and each being observed in 7.14% of WD chromosomes. Haplotype 5-3-2 was associated with most common T3305C Indian WD mutation. Statistically significant deviations were seen for D13S314, D13S301 and D13S316 dinucleotide markers. Test of significance was done by the 2 method for large contingency table, with following results: D13S301 gave a value of 42.12 (P=0.465, 42d.f.), D13S314 yielded a value of 50.02 (P=0.184, 42d.f.) and D13S316 resulted in value of 54.2 (P=0.282, 49d.f.). The genetic heterozygosity for D13S314, D13S301 and D13S316 was observed to be 1.0, 0.81 and 1.0 respectively. These loci showed 0.76, 0.80 and 0.88 expected heterogeneity in these patients respectively. The gene diversity among WD patients was observed to be 0.980.007 (MeanSD). This data has provided an explanation about the association of haplotype with a specific mutation which is utmost important for guiding the identification of mutations in reference to both established and currently unidentified.
(1) Kumar S, Thapa BR, Kaur G, Prasad R. Identification and molecular characterization of 18 novel mutations in the ATP7B gene from Indian Wilson disease patients: genotype. Clin Genet 67(5): 443-445, 2005.

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D85N-KCNE1: A Common single nucleotide polymorphism associated with congenital long QT syndrome. *R.S. Judson[1], B.A. Salisbury[1], M. Pungliya[1], J. Carr[1], J. Hennessey[1], C. Harris-Kerr[1], M. Qi[2], W. Zareba[2], J.L. Robinson[2], A.J. Moss[2], D.J. Tester[3], M.L. Will[3], M.J. Ackerman[3].* 1) Genaissance Pharmaceuticals, New Haven, CT; 2) University of Rochester, Rochester, NY; 3) Mayo Clinic College of Medicine, Rochester, MN.

INTRODUCTION: Rare, often unique, protein-altering mutations in the genes KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 cause approximately 75% of congenital Long QT Syndrome (LQTS). We hypothesized that one or more polymorphisms, protein-altering or not, in the five genes above might contribute to LQTS. We predicted that such contributing polymorphisms would be particularly overrepresented in mutation-negative cases of congenital LQTS relative to non-diseased controls. **METHODS:** The five LQTS-associated channel genes were sequenced for 98 unrelated, white cases with clinically identified LQTS but no clear pathogenic mutation (M-) and for 364 white controls. Hundreds of single nucleotide polymorphisms and several indels were identified. We tested for correlation between case/control status and gene variants ranging from single polymorphisms up to 4-polymorphism haplotypes; permutation testing was used to control for multiple comparisons. **RESULTS:** The common KCNE1 polymorphism D85N was significantly more common among M- cases (11/98, 11%) than in controls (9/364, 2.5%) ($p = 0.0007$). Notably, all 11 D85N+, M- cases were female ($p = 0.03$). No other polymorphism or haplotype was significant after multiple comparisons correction. In an effort to validate these findings, we reviewed data from the FAMILION genetic test for LQTS. Nine (five female) of 95 M- patients carried D85N (9.5%, $p = 0.0045$ vs. controls). **CONCLUSIONS:** The one significant genetic difference between controls and M- cases was overrepresentation of D85N among cases, which we have confirmed in a second case sample. Clinical and functional research have previously implicated D85N as a risk factor for drug-induced torsade de pointes. This congruence of findings strongly implicates D85N-KCNE1 as contributing to the phenotypic presentation of LQTS in some patients. However, further studies are needed to determine whether the D85N has a causative or modifying role.

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Quantitative Analysis of Dystrophin Genes Based on DHPLC : A Highly Efficient and Reliable Duchenne muscular dystrophy Carrier-Screening Test. *M.J. Lee[1], H.W. Lee[1], C.H. Lee[1], B.B. Park[2], J.S. Lee[2].* 1) Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea; 2) Department of Clinical Genetics, Yonsei University College of Medicine, Seoul, Korea.

The dystrophinopathies - the severe type Duchenne muscular dystrophy (DMD) and the milder type Becker (BMD) are the most common inherited disorders of muscle with a prevalence of 1:3500 live male births. Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused in the majority of cases by deletions of the DMD gene and are readily detectable in affected males by multiplex polymerase chain reaction (PCR). However, different approaches must be used for the identification of female carriers, in which deletions are not detectable by PCR, because of the presence of a normal X chromosome. In this study, we show a new, rapid, simple, and highly reliable method for detecting female carrier of DMD by DHPLC. Carrier samples were prepared from patients' family in which patients had shown deletions in their dystrophin gene. Also, we prepared 10 female control individuals from the general population. Carriers have deletion in the dystrophin gene on X chromosome and the amount of PCR product must be half reduced than normal female sample. By DHPLC analysis, we could detect DMD carriers efficiently just by recognizing an only peak that corresponds to a single gene dosage compared to internal standard. The ratio between peaks of carriers and internal controls showed the range from 0.63-0.70. This method is fast, easy and reproducible to detecting gene deletion in DMD carriers and the method might be very helpful for the diagnosis of other diseases that carry a deletion as a mechanism of the diseases such as microdeletion syndrome.

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Comprehensive alpha- and beta-thalassemia genotyping by means of reverse-hybridization teststrips. *H. Puehringer[1], H. Najmabadi[2], E. Baysal[3], W. Krugluger[4], H.Y. Law[5], V. Viprakasit[6], C. Oberkanins[1].* 1) ViennaLab Labordiagnostika GmbH, Vienna, Austria; 2) Genetics Research Center, Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 3) Genetics Department, Al Wasl Hospital, Dubai, UAE; 4) Dept. Clinical Chemistry, Municipal Hospital Rudolfstiftung, Vienna, Austria; 5) Genetics Service, KK Women's and Children's Hospital, Singapore; 6) Dept. Paediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Alpha- and beta-thalassemia (thal) are among the most common inherited diseases throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Mutations in the beta-globin gene, or in one or both of the two alpha-globin genes, are leading to structural abnormalities (e.g. sickle cell anemia) or to haemoglobin imbalance due to the reduced synthesis or complete absence of the respective globin chains. Unlike the prevalence of point mutations in beta-thal, the majority of alpha-thal alleles are derived from single or double gene deletions. We have developed reverse-hybridization assays (StripAssays) for the rapid and comprehensive genotyping of alpha- and beta-thalassemia. The tests are based on multiplex DNA amplification (including gap-PCR) and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each variant. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation. The tests are simple and convenient, and require very small amounts of samples, which is of particular importance for prenatal diagnosis. Although the spectrum of alpha- and beta-thal mutations is known to be highly population-specific, the broad range of variants covered by the StripAssays should make them globally useful diagnostic tools. (oberkanins@viennalab.co.at).

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Analysis of Transcription from D4Z4 Repeats and Satellite DNA in FSHD, ICF Syndrome, Cancer, and Control Cell Populations. *M. Ballestas[1], V. Alexiadis[2], M. Warren[2], C. Sanchez[1], S. Winokur[3], V. Vedanarayanan[4], M. Ehrlich[1].* 1) Human Genetics, Tulane Medical School, New Orleans, LA 70112; 2) Genpathway, San Diego, CA 92121; 3) Dept. Biol. Chem., Univ. Cal., Irvine, CA 92697; 4) Dept. Neurology, Univ. Miss. Med. Sch., Jackson, MS 39216.

There is increasing interest in whether large tandem DNA repeats not associated with known genes are transcribed. Tandem arrays of 3.3-kb D4Z4 repeats at 4q35 containing <11 monomers are diagnostic for facioscapulohumeral muscular dystrophy (FSHD). Because D4Z4 repeats have a putative homeodomain-like ORF downstream of a sequence that acts as a promoter in reporter assays, we looked for transcription of D4Z4 or closely related sequences in various cell types by real-time RT-PCR and by chromatin immunoprecipitation (ChIP) with an RNA polymerase II-specific antibody. We analyzed FSHD and control myoblasts and lymphoblastoid cells; lymphoblastoid cells from ICF patients (immunodeficiency, centromeric region instability, facial anomalies), in which D4Z4 is hypomethylated; several normal tissues; and cancers (ovarian carcinomas and Wilms tumors). Despite in-silico predictions of a gene in D4Z4 repeats in the arrays at 4q35 and 10q26 and in related sequences in acrocentric chromosomes and the cataloging of a D4Z4 cDNA Genbank clone, we found no consistent evidence for transcription of four D4Z4 subregions or closely related sequences in these assays or in ChIP with an antibody to RNA polymerase I. In contrast, we found evidence by both RNA polymerase II ChIP and RT-PCR for very low levels of transcription from pericentromeric satellite 2 sequences in some cancer samples. We conclude that the hypothesized differential transcription of D4Z4 sequences in short, FSHD-diagnostic arrays vs. long arrays is unlikely. Furthermore, it has been proposed that transcription of long heterochromatic regions in eukaryotic cells may generally be involved in maintaining their chromatin structure. However, the very low frequency with which satellite 2 sequences were found to be transcribed in our study does not support this hypothesis for human cells. (Supported in part by NIH grant NS048859, the Muscular Dystrophy Association, & Genpathway.).

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Improved Analysis of FSHD-Linked D4Z4 Repeat Arrays with D4Z4 Probes. *M. Ehrlich[1], K. Jackson[1], P. Camaño González[2], R.J.F.L. Lemmers[3].* 1) Dept Human Genetics, SL31, Tulane Medical Sch, New Orleans, LA; 2) Hospital Donostia, Unidad Experimental, San Sebastián, Guipúzcoa, Spain; 3) Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

The number of tandem copies of the copy-number polymorphic 3.3-kb D4Z4 repeat at 4q35 plays a critical, albeit enigmatic, role in facioscapulohumeral muscular dystrophy (FSHD). Individuals with <8 repeats at one of the allelic subtelomeric 4q arrays usually have the disease while those with 11 up to ~100 almost always do not. In most cases, disease-causing short arrays on 4q can be distinguished from longer 4q arrays and phenotypically neutral, short D4Z4 arrays on 10q26 by pulsed field electrophoresis using p13E-11, which is adjacent to 4q and 10q D4Z4 arrays, as the probe for blot-hybridization to *Eco*RI or *Eco*RI/*Hin*dIII, *Eco*RI/*Bln*I, and *Xap*I digests. This discrimination relies on a *Bln*I site present in canonical 10q, but not 4q, D4Z4 repeats and an *Xap*I site in canonical 4q, but not 10q, repeats. However, deletions of the p13E-11 region in ~3% of individuals complicate the identification of short FSHD-causing D4Z4 arrays on 4q. For these atypical cases and for samples in which the amount of DNA in agarose plugs for PFGE is limiting, such as prenatal specimens, it would be useful to use D4Z4 sequences as the hybridization probe. Furthermore, a D4Z4 hybridization probe aids research on FSHD and analysis of complicated D4Z4 arrays containing translocations between 4q and 10q. The use of a D4Z4-derived probe presents the complication of numerous sequences not at 4q35 and 10q26 that have high homology to D4Z4. We rigorously tested different hybridization conditions and probe sequences that allow 4q and 10q D4Z4 sequences to be distinguished from D4Z4-like sequences elsewhere in the genome. We found conditions that allow probing of a Southern blot with a D4Z4 subfragment to give hybridization essentially only to 4q35 and 10q26 D4Z4 arrays. We present examples of how the use of this probe in addition to the p13E-11 probe can improve molecular diagnosis of FSHD. (Supported in part by NIH Grant R01 NS048859 to M.E.).

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Utility of gross deletion/duplication analysis in Cystic Fibrosis diagnostics. *A. Kammesheidt, T. Vo, K. Schmidt.*
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Cystic Fibrosis (CF) is one of the most common autosomal recessive diseases characterized by chronic pulmonary obstructive disease, exocrine pancreatic insufficiency, elevated sweat chloride levels and male infertility. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), with over 1300 mutations described to date. The distributions and mutation frequency is extremely diverse and only a handful of mutations occur at frequencies greater than 0.1%, whereas most mutations are rare. Even with full gene sequence analysis now available for diagnostic use, clinically affected individual are not always conclusively genotyped. The inability of most PCR based analysis methods to detect gross deletions, duplications and rearrangements has limited diagnostic capabilities. Homozygous gross deletions may be detected in individuals due to the lack of any amplification, but most heterozygous gross deletions remain undetected due to template masking. Recent work suggests that deletions are more abundant in CF than previously thought.

In the present study, 103 samples were screened for deletions/duplications using the multiplex ligation-dependent probe amplification method (MRC, Holland) following full gene sequence analysis with the Ambry Test: CF. 9/103 cases contained deletions or duplications. All deletions were identified in diagnostic cases with positive or borderline sweat chloride levels, in which only 0 or 1 other mutation had previously been identified with the Ambry Test-CF (9/51). Other tested groups included diagnostic cases with 2 identified mutations (n=22), carrier screens with 1 known mutation (n=8), positive newborn screens with 1 or 2 known mutations (n=11) and atypical diagnostics with 1 known mutation (n=11). Gross deletions or duplications were not observed in these groups.

Gross deletion/duplications were present in 18% (9/51) of unresolved diagnostic cases in this sample set. The data support that gross deletion/duplication analysis is an essential reflex test to improve detection rates in symptomatic individuals undergoing CFTR genotyping.

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A preferential association of CF mutations with CFTR-M470 allele. *C. Bombieri[1], F. Pompei[2], B.M. Ciminelli[2], A. Bonizzato[3], A. Holubova[4], P. Zorzi[1], A. Begnini[1], C. Ciccacci[2], V. Vavrova[5], C. Castellani[3], M. Macek jr[4], G. Modiano[2], P.F. Pignatti[1].* 1) Section Biol & Genetics, Univ Verona, Verona, Italy; 2) Dpt. of Biology, Univ. Roma-Tor Vergata, Italy; 3) Veneto Regional CF Center, Verona Hospital, Italy; 4) Inst Biology and Medical Genetics, Czech Republic; 5) Pediatric Clinic, Charles Univ. Prague, Czech Republic.

In a previous study on European non-CF individuals, we found a preferential association of random CFTR gene mutations with the M allele of the M470V common polymorphism. Therefore, we hypothesized that CFTR alleles with the M variant should have an increased risk of carrying a CF-causing mutation. Last year we reported on this association in a first group of Italian CF patients. Now we wish to report the characterization of a larger series of Italian CF patients, and a confirmation in Czech patients. Patients and their parents were selected for the presence of at least one non-F508del mutation (as F508del is associated with M) in the CF child, after screening for the most common CF mutations in the population. In Italian CF patients 221/272 (81%) CF-non-F508del mutations were detected on the M allele. In Czech patients, this figure was 53/86 (62%). As the frequency of the M allele in the non-CF population is 39%, the difference is highly significant. The observed population difference is related to the frequency of some population-characteristic mutations: in particular, dele2,3-21kb in the Czech population only (16/86 on the V allele), and R1162X in the Italian population only (35/272 on the M allele). In the Italian population, MM individuals (15% in the population) will have a relative risk of carrying a CF mutation about 15X that of a VV individual (38% in the population). In conclusion, population screening for the M470V polymorphism may help modify the estimate of the risk to be a carrier of a CF mutation, and further analysis could be suggested for individuals at increased risk.

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Two previously unreported COL4A3 mutations in a patient with autosomal recessive Alport syndrome. *G.L. Barnett[1], R. Baliga[2], C.A. Friedrich[1].* 1) Dept Prev Med, Div Med Genet,; 2) Ped Nephrology, Univ Mississippi Medical Ctr., Jackson, MS.

A 14-year-old African-American female with longstanding bilateral hearing loss developed hypertension, hyperkalemia, elevated BUN and creatinine, hematuria, anemia, proteinuria, and a decreased erythropoetin level. Neither of her parents and neither of her sisters had hearing loss, renal disease or hypertension. A distant older relative with hypertension and diabetes mellitus required dialysis but no other relatives were known to have renal disease or hearing loss. Renal biopsy and electron microscopy revealed segmental effacement of podocyte foot processes and changes suspicious for hereditary laminated basement membrane disease. DNA sequencing of COL4A3 was performed by Dr. Mato Nagel (Weisswasser, Germany) and showed two mutations in exon 49. R1496X (CGA>TGA) at codon 1496 and R1516X (CGA>TGA) at codon 1516 are both premature termination codons that have not been reported previously. The most likely explanation is each of these mutations is on a different allele but family studies are needed to confirm this. Presymptomatic diagnosis may be an option for her siblings.

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Report of a novel mutation in an Iranian individual affected with Marfan syndrome. *R. Karimi-Nejad[1], V. Hadavi[1], B. Mahshidfar[1], J.S. Saldivar[2], M.H. Kariminejad[1], H. Najmabadi[1].* 1) Kariminejad-Najmabadi Pathology & Genetics Center, 14665/154, Tehran,Iran; 2) Clinical Molecular Diagnostic Laboratory, City of Hope National Medical Center and Beckman Research Institute, Duarte, California, US.

Marfan syndrome, which was originally described in the 19th century, is an autosomal dominant connective tissue disease associated with mutation in the fibrillin-1 gene located on chromosome 15 (locus 15q21.1). A 31 years old male affected with Marfan syndrome was referred to our center. According to family history, there was no one else with the disease. The phenotype of the individual assigned affected status satisfied the established diagnostic criteria for MFS. Progressive high myopia and bilateral lens subluxation have been diagnosed since he was 5, a tall stature and tall extremities, pectus excavatum, high arched palate, a little joint laxity, but no arachnodactyly were noted. Dilated aortic root was diagnosed and MVP found in echocardiography. All coding exons and associated intron junctions of the FBN1 (65 exons) gene were analyzed by direct DNA sequence analysis using an automated fluorescent sequencer. Confirmation of the mutation detection was carried out by sequencing in the opposite direction. The results of sequencing showed deletion of CAC and insertion of 12 bp at nucleotide -5 in intron 50 (IVS 50-5 delCACinsTCATTAATAGAT). This genetic alteration inserts a putative alternative acceptor splice site junction in intron 50. Based on its sequence, it is predicted to insert 2 bp into the current open reading frame, which would result in a frameshift mutation. Thus it is very likely that this alteration results in a major disruption in the structure of the FBN1 protein and is predicted to be deleterious to the normal function of the protein. However, the possibility that this mutation represents a rare polymorphism cannot be completely ruled out. The individual is heterozygous for this alteration, having an apparently “normal” or “wild” type copy of the FBN1 gene along with the altered copy. To our knowledge, this alteration has not been previously reported.

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Deletion screening of the *FOXL2* and *FOXC1* region involved in BPES and Axenfeld-Rieger malformations using high-resolution tiling path BAC microarrays. *L. Dejager[1], B. Menten[1], D. Beysen[1], J. Vandesompele[1], E. Michels[1], B.P. Leroy[1,2], B. Lorenz[3], F. Meire[2], P. Kestelyn[2], T. de Ravel[4], Y. Arens[5], S. De Jaegere[1], A. De Paepe[1], F. Speleman[1], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium; 3) Ophthalmogenetics, Klinikum der Universität Regensburg, Regensburg, Germany; 4) Center for Medical Genetics, Leuven University Hospital, Leuven, Belgium; 5) Center for Medical Genetics, University of Maastricht, Maastricht, The Netherlands.

MLPA and microsatellite analysis recently enabled us to detect genomic deletions that either encompass or are located in close vicinity of the forkhead transcription factor gene *FOXL2* in families with blepharophimosis syndrome (BPES). These deletions account for 16% of all molecular defects we found in BPES. The extragenic deletions are located upstream and downstream of the transcription unit of *FOXL2* and represent a new mutational mechanism for BPES.

In addition, changes in gene dosage of *FOXC1*, another forkhead transcription factor gene, are known to result in a spectrum of dominant disorders involving malformations of the anterior segment of the eye (e.g. Axenfeld-Rieger anomalies). In this study, we identified 4 deletions encompassing *FOXC1* in patients with Axenfeld-Rieger malformations using MLPA.

We developed a 2.9 Mb and 3.1 Mb tiling path BAC array for breakpoint delineation in known deletion patients and for detection of deletions in patients in which no genetic defect was found so far. ArrayCGH allowed to delineate the breakpoints in 16 known deletion patients with BPES and 4 patients with Axenfeld-Rieger anomalies respectively. Interestingly, a novel extragenic deletion was identified upstream of *FOXL2* in a sporadic BPES patient.

In conclusion, this study shows that arrayCGH with a tiling path array for the *FOXL2* and *FOXC1* region is a robust and efficient approach to identify and characterize subtle genomic rearrangements in these two regions.

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Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia: a French-Italian study. *G. LESCA[1], C. OLIVIERI[2], N. BURNICHON[3], F. BOUTITIE[4], F. ABBAS[4], F. COULET[5], M. RABILLOUD[4], A. CALENDER[1], S. GIRAUD[1], F. SOUBRIER[5], C. DANESINO[2], E. BUSCARINI[6], H. PLAUCHU[3], French Italian Rendu-Osler Network.* 1) Laboratoire de Genetique, Hopital Edouard Herriot, Lyon, France; 2) Genetica Medica, Universita di Pavia, Italy; 3) Service de Genetique, Hotel-Dieu, Lyon, France; 4) Service de Biostatistique, Lyon, France; 5) Laboratoire de Genetique, Hopital Pitie-Salpetriere, Paris, France; 6) 6Gastroenterologia ed Endoscopia Digestiva, Ospedale Maggiore, Crema, Italy.

Hereditary Hemorrhagic Telangiectasia is an autosomal dominant disorder characterized by arteriovenous malformations (AVM) with cutaneomucous (telangiectases), pulmonary (PAVM), hepatic (HAVM) and cerebral (CAVM) involvement. We recruited 342 patients, 93 having a mutation in ENG (HHT1) and 249 in ALK1 (HHT2), respectively, through the Rendu-Osler Network in France and Italy. Epistaxis occurred earlier in HHT1 (12yrs) than HHT2 (19yrs), with incomplete penetrance for HHT2. The number and repartition of cutaneous telangiectases did not differ according to the type. The prevalence of PAVMs was highly significantly different between HHT1 (53%, n=83) and HHT2 (15%, n=175). PAVMs were found in 25% of the patients who underwent systematic screening (n=199), but with a higher occurrence in HHT1 (54%, n=50) than in HHT2 (13%, n=149). The prevalence of HAVMs was globally high (57%, n=179) and significantly higher in HHT2 (61%, n=171) than in HHT1 (43%, n=47). Severe HAVMs requiring liver transplantation were only seen in the HHT2 group. Intestinal telangiectases were found in 38% of the patients who underwent systematic screening (n=57), with no difference between HHT1 and 2. CAVMs were found in 1.5% of the patients after a clinical manifestation and in 6% after systematic screening (n=72), without difference according to the genotype. Severe infections (excluding cerebral abscess) were reported in both groups without noticeable difference. When considering the mutation type, there was a trend toward an association between ALK1 missense mutations and hepatic involvement, as well as between PAVMs and small deletion/duplication of ENG.

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Beta-globin cluster linked polymorphisms strongly associated with the variability of disease severity in Thai thalassemia patients with ^{0}/HbE genotype. *Q. Ma[1], K. Abel[2], J. Whitacre[2], V. Angkachatchai[2], W. Makarasara[3], O. Sripichai[3], P. Fucharoen[3], S. Fucharoen[3], A. Braun[2], L.A. Farrer[1].* 1) Boston University, Boston, MA; 2) Sequenom Inc., San Diego, CA; 3) Mahidol University, Thailand.

Thalassemia patients possessing both HbE and ^{0}mutant alleles display remarkable variability in disease severity. We evaluated the contribution of polymorphisms within the -globin cluster to variation in disease severity by genotyping 70 SNPs in this region with an average spacing of 1,066bp in groups of 209 mild and 327 severe unrelated patients from Thailand with ^{0}-thalassemia/HbE disease and normal -globin genes. Eleven SNPs uninformative in this sample were excluded from further analysis. All statistical analyses were adjusted for age, gender, and geographic region. Logistic regression was used to investigate the association between each SNP and disease severity. Thirty-nine SNPs spanning the LCR region and A gene showed strong association with disease severity. The strongest association was observed with the *XmnI* polymorphism located 158 upstream of the G gene (global p=1.4E-12). Subjects with the CC genotype were 4.1 times more likely than CT subjects (p=2.4E-07) and 34.5 times more likely than TT subjects (p=1.7E-12) to have severe disease. Haplotype analysis of five tagging SNPs (*HincII-3′yb*; *HindIII-Gg*; *XmnI*; *HincIIE*; *rs4601817*) showed that disease severity was less among subjects with the haplotype ATT-T (freq=0.4;p=6.3E-16) and increased among subjects with haplotypes GGCAC (freq=0.48;p=5.8E-07) and GGCAT (freq=0.09;p=5.2E-04). Haplotype analysis of the *XmnI* site and the -globin mutation among 536 ^{0}/HbE patients and 50 subjects homozygous for the HbE mutation revealed that T allele of *XmnI* was nearly always in cis with the HbE allele(haplotypes frequencies: T-HbE=0.39; T-^{0}=0.04; C-^{0}=0.43; C-HbE=0.14). Linear regression analysis showed the XmnI genotypes TT and CT were associated with increased expression of fetal hemoglobin (HbF) in both the mild (p=0.004) and severe (p=9.3E-09) patient groups. In summary, our study showed that *XmnI* and other SNPs in the -globin cluster are associated with disease severity and expression of HbF in Thai patients with ^{0}-thalassemia/HbE disease.

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Familial hemophagocytic lymphohistiocytosis: Mutation spectrum, genotype/phenotype correlation, and functional analysis of *PRF1, UNC13D, STX11,* and *RAB27A*. *H.C. Hennies[1], K. Beutel[2], S. Kolberg[3], R. Schneppenheim[2], H. Kabisch[2], G. Janka[2], U. zur Stadt[2].* 1) Cologne Center for Genomics, Dermatogenetics, Univ of Cologne, Germany; 2) Dept of Pediatric Hematology and Oncology, Univ Medical Center of Hamburg, Germany; 3) Gene Mapping Center, Max Delbrück Center for Molecular Medicine, Berlin, Germany.

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disease affecting young children. It presents as a severe hyperinflammatory syndrome with activated macrophages and T lymphocytes. Mutations in the perforin 1 gene (*PRF1*) were reported in FHL-2 in 15-50% of all cases. Defective granule exocytosis caused by mutations in the hMunc13-4 gene (*UNC13D*) has been described in FHL-3. FHL-4 patients have mutations in *STX11*, a t-SNARE involved in intracellular trafficking. We have analysed a large group of 63 unrelated patients with FHL from different geographic origins for mutations in *STX11*, *PRF1*, and *UNC13D*. We identified mutations in 38 samples, 20 in *PRF1*, 12 in *UNC13D*, and 6 in *STX11*. Of 32 patients from Turkey, 14 had mutations in *PRF1*, 6 in *UNC13D*, and 6 in *STX11*. The mutation Trp374X in *PRF1* was found in twelve patients from Turkey and was associated with a very early onset of the disease below the age of three months in all cases. In contrast, only 7/23 patients from Germany showed mutations in either *PRF1* or *UNC13D*. Thus FHL-2, FHL-3, and FHL-4 account for 80% of the HLH cases of Turkish origin and for 30% of German patients. Using a mammalian two-hybrid system we have now shown that the missense mutations Ala87Pro in Rab27a, which was found in a patient with FHL-related Griscelli syndrome type 2, and Leu403Pro in hMunc13-4 each prevented the formation of a stable hMunc13-4/Rab27a complex in vitro. Our findings demonstrate extensive genetic and allelic heterogeneity in FHL and delineate an approach to functionally characterize missense mutations in *RAB27A* and *UNC13D*.

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A genetic test of the potential role of brain-derived neurotrophic factor (BDNF) in modulating Huntington'fs disease pathogenesis. *S. Kishikawa[1], R.H. Myers[2], S. Williamson[2], T. Gillis[1], M. Hakky[1], M.E. MacDonald[1], J.F. Gusella[1].* 1) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Charlestown, MA; 2) Department of Neurology, Boston University School of Medicine, Boston, MA.

Huntington'fs disease (HD) is an autosomal dominant neurodegenerative disorder, caused by an expanded polyglutamine tract in the huntingtin protein (htn). The size of the repeat accounts for up to 70% of variance in age at onset, but the remaining variation is strongly heritable, suggesting that genetic modifiers affect the pathogenic process. BDNF is a prosurvival factor that is necessary for survival of striatal neurons in the brain. BDNF also has been suggested to have a specific role in the HD pathogenic process, because htn upregulates transcription of BDNF and promotes BDNF transport with Huntington-associated protein-1 and the p150 (Glued) subunit of dynactin. To test this hypothesis, we analyzed two BDNF SNPs for association with onset age in HD: rs6265 is a functional SNP located in the coding region (V66M) that alters intracellular trafficking and activity-dependent secretion of BDNF. BDNF270 is located in the 5'-flanking region of BDNF. Both have been reported to show genetic association with behavioral disorders. We genotyped six hundred and thirty one (279 unrelated singletons and 352 siblings) HD affected individuals with extremely young or extremely old onset ages (0.5 S.D. from expected repeat adjusted onset age) and 83 additional HD affected persons by direct sequencing or by TaqMan methods. In this dataset, neither rs6265 (p=0.73) nor BDNF270 (p=0.99) was associated with onset age, in models controlling for the HD repeat size. These results, showing that genetic alteration of BDNF activity does not significantly alter age at neurologic onset, suggest that BDNF does not play a major role in influencing the HD pathogenic process that leads to disease onset.

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Genotype-phenotype correlation in NF1 microdeletion syndrome: CDKR1 and OMG mutations in mental retarded patients and genes specifically expressed in embryonic heart. *M. Venturin[1], S. Moncini[1], V. Villa[1], S. Brunelli[3], S. Russo[2], M.T. Bonati[2], L. Larizza[1,2], P. Riva[1].* 1) Department of Biology and Genetics, Medical Faculty, University of Milan, Milan, MI, Italy; 2) Istituto Auxologico Italiano, Milan, MI, Italy; 3) Stem Cell Reasearch Institute, HSR, Milan, MI, Italy.

Mental retardation (MR) and cardiovascular malformation (CVM) are significantly present in NF1 patients (pts) with microdeletion syndrome, compared to classical NF1 pts, as a result of 17q11.2 region haploinsufficiency. We identified in the NF1 deleted region the CNS developmental genes CDK5R1 and OMG, as candidate for MR susceptibility. We screened 100 pts affected by unspecific MR for mutations in OMG and CDK5R1 coding regions and in 5/3 UTRs, in which latter we predicted several transcription/translation regulatory elements. Four novel mutations and 2 novel polymorphisms for CDK5R1 and 3 novel mutations for OMG were detected, including 2 missense changes (c.323C>T; A108V in CDK5R1, and c.1222A>G; T408A in OMG), 1 synonymous variant (c532C>T; L178L in CDK5R1), 4 variants in CDK5R1 3UTR and 2 changes in OMG 5UTR. Prediction of mRNA and protein secondary structures revealed that changes in the mutated 2254G CDK5R1 3UTR and OMG 408A gene product, lead to putative structural alterations. We didnt found in 17q11.2 known genes involved in heart development. Expression analysis of 9 genes within the NF1 deleted region showed that CENTA2, SUZ12, C17orf40 are mainly expressed in human fetal heart and mouse embryonic heart during the early stages of development. In situ hybridization on whole mouse embryos and sections from 8.25 to 14 dpc evidenced that CENTA2 is expressed in developing heart starting from 9.25 dpc. These studies allowed us to identify mutations in CDK5R1 and OMG, which pathogenetic role will be investigated and suggested that CENTA2 is involved in heart development and possibly in CVM. Clinical/genetic analyses of NF1 microdeletion syndrome may be functional to disclosing genes involved in complex diseases, enhancing genotype-phenotype correlation.

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The Jackson Laboratory Repository: Mouse Models of Human Disease. *S.F. Rockwood, L.R. Donahue, D.B. Lane, M.T. Davisson.* The Jackson Laboratory, Bar Harbor, ME.

Mouse models of human disease continue to fulfill an essential role in facilitating the development of therapeutic applications. To ensure the greatest possible access to these models, The Jackson Laboratory Repository was established to serve as a centralized facility for the purpose of developing, collecting, distributing and cryopreserving mouse models for the international biomedical research community. Approximately 100-150 new strains are added annually to the hundreds of unique mouse strains that comprise the largest collection of characterized mouse strains available to researchers today. Models newly imported and/or developed for distribution have applications in studies focusing on Spinal Muscular Atrophy (SMA), Rett syndrome, Cystic Fibrosis, Diabetes, Huntingtons disease, Alzheimers disease, craniofacial disorders, kidney disease, vision and hearing disorders and spondyloepiphyseal dysplasia (SED). Supplementing these strains are a wide variety of Cre-expressing strains (constitutive or inducible) and a growing number of targeted mutants harboring loxP-flanked genes that are useful for generating tissue-specific mutants. An on-line resource is available (www.jax.org) allowing researchers to retrieve information related to strains maintained in the Repository. On-line strain datasheets typically include a brief phenotype description, strain development, husbandry information, licensing requirements and a list of related references. Donating a strain to the Repository fulfills the requirements for sharing of mice in accordance with NIHs policy for the sharing of research reagents. Researchers wishing to have strains considered for inclusion in the Repository may submit their strains using the form available at The Jackson Laboratory web site: http://www.jax.org/grc/index.html. The Jackson Laboratory Repository is supported by the National Center for Research Resources, the National Institute on Aging, the National Cancer Institute, The Howard Hughes Medical Institute, the Ellison Foundation, and private donations from several charitable foundations. (P40 RR01183, RR09781, RR16049, N01 HD53230, R01 Ey015073, P30 CA34196).

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Proximal Inclusion body myopathy, Paget Disease of the Bone and Frontotemporal Dementia (IBMPFD): a model for adult onset complex traits. *V. Kimonis[1], S. Ramdeen[1], S. Mehta[1], C. Smith[2], K. Boycott[3], R. Schröder[4], G. Watts[1].* 1) Div Gen/Metabolism, Child Hosp, Harvard Medical Sch, Boston, MA; 2) Dept of Neuro,Univ. of Kentucky, Lexington, KY; 3) Univ. of Calgary, Alberta Childrens Hospital, Calgary, AB, Canada; 4) Dept of Neurology, Univ. Hosp, Bonn, Germany.

IBMPFD is a dominant progressive disorder that maps to chromosome 9p21.1-p12 and associated with mutations in VCP (valosin-containing protein), a member of the AAA-ATPase superfamily. Among 133 individuals in 20 unrelated families, 93(70 %) had myopathic weakness at a mean age of 42 (Range 3-61 y). PDB was identified in 51(38 %) at a mean age of 42 years (Range 31-61 y) typical distribution including the spine, pelvis, scapulae and skull. FTD was seen in 32(24 %) with a mean age of diagnosis of 55 years. Rimmed vacuolar inclusion bodies were present in 35 % of muscle biopsies. Histological studies reveal filamentous inclusion bodies in muscle cytoplasm and nucleus, Pagetic osteoclasts and brain, which stain positive for VCP, ubiquitin, phosphorylated tau and -amyloid, these deposits being similar to those seen in Alzheimer disease. On further analysis of the data for genotype-phenotype correlations, the R155C individuals had an earlier age of onset for the myopathy and PDB ($p<0.05$) when compared to R155H ($p<0.05$) and other mutations. The A232E mutation in a small family involving the AAA ATPase domain not surprisingly was associated with a more severe phenotype and early demise before the onset of dementia would be expected. Interestingly we found that inheriting 1 or 2 alleles of APOE4 was associated with an increased predisposition for frontotemporal disease ($p<.01$) and women were twice as likely to have dementia. VCP is associated with a variety of cellular activities, including cell cycle control, membrane fusion and the ubiquitin-proteasome degradation pathway. The majority of mutations in VCP cluster in the N terminus, and have been shown to interfere with poly-ubiquitin and co-factor binding thus permitting potential therapeutic targeting strategies for this complex disorder.

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Myotonia Congenita in a large consanguineous Bedouin- Muslim family; A unique clinical presentation and identification of a novel mutation in the (CLCN1) Chloride Channel gene. *A. Shalata.* Simon Winter Ins.Human Genetic, Bnai-Zion Medical Center, Haifa, Israel.

Myotonia is the term given to delayed relaxation of skeletal muscle after voluntary contraction. In most situations myotonia is most marked after initial muscle contraction, and usually abates after repeated muscle activity (the warm-up phenomenon). Congenital myotonia, the most common form of non-dystrophic forms of myotonia, may show both dominant (Thomsen disease) and recessive (Becker disease) modes of transmission, both of which may be caused by mutations in CLCN1(7q35), the gene encoding the major skeletal muscle chloride channel. Mutations in this gene may cause myotonic contractions in humans, mice, goats and dogs. Today, more than 80 different mutations have been identified in the CLCN1 gene in both types of human myotonia congenita, in various ethnic groups. We describe herein, to the best of our knowledge, the clinical characterization and the molecular analysis of the first Arab family of Bedouin-Muslim origin, affected with myotonia congenita. We identified a novel mutation, the G190S mutation, located in a conserved CLC1-chloride channel motif which is transmitted in a dominant mode of inheritance with incomplete penetrance.

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Identification of novel mutations in *MMP20* and *ENAM* underlying amelogenesis imperfecta. *S. Hart[1], D. Ozdemir[2], O.K. Ryu[3], S.J. Choi[3], E. Firatli[2], M. Ozdemir-Karatas[2], G. Aren[2], N. Piesco[4], T.C. Hart[3].* 1) Office of the Clinical Director, NHGRI, Bethesda, MD; 2) School of Dentistry, Istanbul University, Istanbul Turkey; 3) Section of Craniofacial and Dental Genetics, NIDCR, Bethesda, MD; 4) University of Pittsburgh, School of Dental Medicine, Pittsburgh, PA.

The amelogenesis imperfecta (AI) are a group of disorders that affect enamel formation. Mutations in 3 genes have been reported to cause autosomal hypomaturation (*MMP20, KLK4*) or hypoplastic forms of AI (*ENAM*). We analyzed 25 Turkish families segregating autosomal AI for mutations in the *MMP20* and *KLK4* (hypomaturation phenotype) or *ENAM* (hypoplastic phenotype) genes. Of the 15 hypomaturation families, 1 family was found to have a novel (g.16250T>A) *MMP20* mutation that changes the conserved active site His226 residue of the zinc catalytic domain to Gln (p.H226Q). Zymogram analysis confirmed that this missense mutation abolished MMP20 proteolytic activity. This represents the second *MMP20* mutation described. Of the 10 families segegating hypoplastic AI, *ENAM* mutations were found in 2. A novel transversion mutation (g.12663C>A; p.S246X) was found in a family segregating local hypoplastic AI as a dominant trait. In the second family segregating generalized thin hypoplastic AI, affected individuals were compound heterozygotes for a novel insertion mutation (g.12946_12947insAGTCAGTACCAGTACTGTGTC) and a previously described insertion mutation (g.13185_13186insAG). Heterozygous carriers of either insertion had a localized enamel pitting phenotype, substantiating that enamel phenotypes of *ENAM* mutations may be dose dependent with generalized hypoplastic AI segregating as a recessive trait and localized enamel pitting segregating as a dominant trait. This brings to seven the total number of *ENAM* mutations reported and represents the second mutation associated with pitting in the carrier state. These results suggest that while *MMP20* and *ENAM* mutations are responsible for some cases of AI in Turkey, other genes as yet unknown are responsible for the majority of cases.

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Prevalence and distribution of exonic mutations in Interferon Regulatory Factor 6 (IRF6) identified in two large cohorts with Van der Woude syndrome. *R. de Lima[1], M. Ghassibe[2], N.K. Rorick[3], S.A. Hoper[3], L. Katz[3], D.J. Schipper[3], M.J. Dixon[5], U. Hehr[4], J.G. Compton[6], M. Vikkula[2], A. Richieri-Costa[7], D. Moretti-Ferreira[1], J.C. Murray[3], B.C. Schutte[3].* 1) Genetics, UNESP, Botucatu, Sao Paulo, Brazil; 2) University of Louvain, Brussels, Belgium; 3) Pediatrics, University of Iowa, Iowa City, IA; 4) Humangenetik, Regensburg, Germany; 5) University of Manchester, Manchester, England; 6) GeneDx, Gaithersburg, MD; 7) USP, Bauru, Sao Paulo, Brazil.

Mutations in IRF6 cause Van der Woude (VWS) and Popliteal Pterygium syndromes (PPS), two related orofacial clefting disorders. IRF6 belongs to the IRF family of nine transcription factors. Both VWS and PPS display an autosomal dominant pattern of inheritance with high penetrance but variable expressivity. The phenotype of VWS includes pits in the lower lip, clefts of the lip and/or palate and hypodontia. In addition to these features, PPS includes webbing of the lower limbs, syndactyly of the toes and digits, ankyloblepheron, oral synechia, and genital abnormalities. We performed direct sequence analysis on the exons of IRF6 on samples from two large geographically defined cohorts, one from Brazil (113 VWS and 1 PPS) and one of mixed origin (197 VWS and 36 PPS). In both cohorts, we identified mutations in IRF6 exons in 69% of all families with VWS, and 97% of families with PPS. The distribution of VWS-causing mutations was not random, with exons 3, 4, 7, and 9 accounting for 80%. In total, we identified 87 protein truncation mutations, scattered throughout the gene, and 127 missense mutations that are concentrated in the DNA-binding and protein-binding domains. With 50% of the samples sequenced from the CEPH diversity panel, none of these mutations have been observed. In addition, PolyPhen and SIFT analyses of the missense mutations suggest that they are more likely to be damaging to gene function than all possible missense mutations ($p<.001$). This extensive mutation screen of IRF6 will assist clinicians to provide a DNA diagnosis for patients with orofacial clefts and assist researchers to identify genotype-phenotype and structure-function relationships.

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Search for protein interactors of ribosomal protein S19, mutated in Diamond Blackfan Anemia. *I. Dianzani[1], A. Chiocchetti[1], L. Gibello[1], A. Carando[2], P. Secco[1], A. Biava[1], A. Aspesi[1], U. Dianzani[1], U. Ramenghi[2], S. Orrù[3], M. Angelini[4], M. Caterino[3], M. Ruoppolo[3], F. Loreni[4], C. Santoro[1].* 1) Dept Medical Sciences, Univ Piemonte Orientale, Novara; 2) Dept Pediatric Sciences, Univ.Torino; 3) Dept.Biochemistry and Biotechnology, Univ.Federico II, Napoli; 4) Dept of Biology,Univ.Tor Vergata, Rome.

Diamond Blackfan Anaemia (DBA) (MIM 205900) is a congenital disease characterized by defective erythroid progenitor maturation. Patientsbone marrow progenitors do not respond to eryhtropoietic growth factors, such as EPO. Mutations in the gene encoding for ribosomal protein (RP) S19 account for 25% of patients. The link between defective erythropoiesis and rps19 is still unclear. Two not mutually exclusive hypotheses have been proposed: altered protein synthesis and loss of unknown extraribosomal functions. Several ribosomal proteins have been shown to have a second function besides their structural role in the ribosome. We have used two different approaches to search for proteins interacting with RPS19. Yeast two hybrid screening has been used to screen a human liver cDNA library obtained at 19-24 weeks of gestation, when hepatic erythropoiesis is efficient. We have found that RPS19 binds PIM-1, an ubiquitous serine-threonine kinase whose expression can be induced in erythropoietic cells by several growth factors, such as EPO. The PIM-1/RPS19 interaction was demonstrated both in vitro and in living cells and lead to phosphorylation of RPS19 in an in vitro kinase assay. We also show that in human 293T cells PIM-1 interacts with ribosomes and may be involved in translational control. Three DBA-associated RPS19 mutations alter the binding between RPS19 and PIM-1. An LC/MS/MS approach was performed on the eluate obtained after affinity purification using a GST-RPS19 resin on a lysate from K562 cells. Twenty-six interactors, all nucleolar, were identified. In conclusion, our data show that RPS19s main roles are in ribosome biogenesis and translation. A link between erythropoietic growth factor signaling and RPS19 has been identified for the first time: it may lead to translational control.

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Two Novel *KCNQ2* Mutations in Benign Familial Neonatal Convulsions. *A. Shankar[1], J. Hunter[1], S. Maljevic[2], A. Siegel[1], L. Olson[2], B. Weissman[2], P. Holt[2], H. Lerche[2], A. Escayg[1].* 1) Dept. of Human Genetics, Emory University, Atlanta, GA; 2) Departments of Neurology and Applied Physiology, University of Ulm.

Benign familial neonatal convulsions (BFNC) is a rare autosomal dominant epileptic disorder that results from mutations in two voltage-gated potassium channels, *KCNQ2* and *KCNQ3*. Two BFNC families were identified at the Emory University Epilepsy Clinic. Mutation screening of *KCNQ2* and *KCNQ3* was performed in order to identify the disease-causing mutations. In each family, a novel heterozygous single base substitution was found in the coding region of *KCNQ2*. In Family 1, the nucleotide substitution C740A was identified in the five affected family members. This substitution results in the truncating mutation S247X, which generates a protein that is 37% shorter at the C-terminal end. In Family 2 the nucleotide substitution C365T, leading to the amino acid substitution S122L, was detected in the three affected members. S122L was not observed in 800 control chromosomes. The S122L mutation was functionally expressed in *Xenopus* oocytes and analyzed using the two-microelectrode voltage clamp. Comparison of the biophysical properties of the mutant channel with wildtype *KCNQ2* revealed a 10 mV depolarizing shift in the voltage dependence of activation, as well as slowing of activation upon depolarization. Co-expression of the S122L mutant with *KCNQ3* yielded currents with about 10-fold increased current amplitudes, consistent with functional *KCNQ2*/*KCNQ3* heteromers. In comparison to wildtype channels, these currents were characterized by a 5 mV shift of voltage-dependent activation and slowing of activation upon depolarization. These results demonstrate the significance of the S2 segment in the function of *KCNQ2* channels and suggest that the seizures in Family 2 arise from a reduction in the potassium currents that regulate membrane potential at the sub-threshold range.

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MED25 transcription activator is mutated in CMT2B2, carries a SH3 recognition motif and is coordinately expressed with Pmp22 in CMT1A animal models. *B. Rautenstrauss[1], A. Leal[1, 2], K. Huehne[1], H. Sticht[3], F. Bauer[3], P. Berger[4], U. Suter[4], G. Del Valle[2], R. Barrantes[2], M. Saifi[5], J. Lupski[5], B. Neundoerfer[6], M. Meisterernst[7], M. Berghoff[8], C. Berghoff[8], D. Heuss[6], M. Sereda[9], G. Zu Horste[9], K. Nave[9], A. Reis[1].* 1) Institut of Human Genetics, Erlangen, Germany; 2) School of Biology and INISA,San Jose, Costa Rica; 3) Institute of Biochemistry, Erlangen, Germany; 4) Institute of Cell Biology, ETH Zurich, Switzerland; 5) Baylor College of Medicine, Houston TX, USA; 6) Department of Neurology, Erlangen, Germany; 7) GSF, Munich, Germany; 8) Department of Neurology, Muenster, Germany; 9) MPI of Experimental Medicine, Göttingen, Germany.

We identified an A335V mutation in the MED25 (ARC92/ACID1) gene in an extended Costa Rican family with autosomal recessively inherited Charcot-Marie-Tooth (CMT) neuropathy linked to the CMT2B2 locus in chromosome 19q13.3. MED25, also known as ARC92 and ACID1, is a subunit of the human activator-recruited cofactor (ARC), a family of large transcriptional coactivator complexes related to the yeast Mediator. MED25 was identified by virtue of functional association with the activator domains of multiple cellular and viral transcriptional activators. Its exact physiological function in transcriptional regulation remains obscure. The A335V missense amino acid substitution is located in a proline rich region with high affinity for SH3 domains of the Abelson type and represents a novel transcription activation site. The mutation causes a decrease in binding specificity leading to the recognition of a broader range of SH3 domain proteins. Furthermore, Med25 expression correlates with Pmp22 gene dosage and expression in transgenic mice and rats. Progesterone increases MED25 expression coordinatively with Pmp22. After permanent sciatic nerve transsection, without allowing nerve regeneration, Pmp22 transcript levels were strongly reduced while Med25 expression was only moderately decreased. These results establish the molecular etiology of CMT2B2 and suggest a potential, more general role of MED25 in gene dosage sensitive peripheral neuropathy pathogenesis.

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CRYAB Mutation Causes Autosomal Dominant Posterior Polar Cataract in a Chinese Family. *M. Liu[1], T. Ke[1], Z. Wang[2], Q. Yang[1], Q. Wang[1,3].* 1) Human Genome Research Center, Wuhan, Hubei, China; 2) People's hospital of Linyi, Shangdong, China; 3) Center for Molecular Genetics, Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation,Cleveland,Ohio,USA.

Congenital cataract is a leading visual disease, and is responsible for approximately one third of blindness in infants. Inherited isolated (nonsyndromic) cataract represents a significant proportion of cataracts. Six different crystalline genes, including CRYAB that encode alpha-B (aB)-crystallin, have been identified as the causes of autosomal dominant cataract. To date, only one mutation (450delA) in CRYAB has been identified in one cataract family from U.K., and one other mutation (R120G) was identified in a French family with desmin-related myopathy. In this study, we studied a four generation Chinese family with non-syndromic posterior polar cataract. A genome-wide scan and haplotype analysis mapped the disease gene to chromosome 11q22-22.3 with a maximum LOD score of 3.85, where CRYAB is located. SSCP and directly DNA sequence analysis of the complete coding region and exon-intron boundaries of CRYAB revealed a heterozygous CT transition at nucleotide 58, resulting in a novel c.58 CT (p. Pro20Ser) mutation. The p.Pro20Ser mutation co-segregated with all affected individuals, and was not present in unaffected members in the family and 200 normal controls. The mutation occurs at the evolutionally conserved residue Pro20 in the crystallin_N region of CRYAB. Our study identifies the second mutation in CRYAB associated with congenital isolated cataract, and demonstrates that the mutations in CRYAB cause isolated congenital posterior polar cataract.

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Novel DSPP Mutation Causes dentinogenesis imperfecta type II. *X. Zhang[1], L. Chen[2], M. Liu[1], Q. Wang[1,3].* 1) Center for Human Genome Research, Huazhong University of Science and Technology,P.R.China; 2) Department of Bioenjineering, Henan Urban College of Engineering, P. R. China; 3) Center for Molecular Genetics, Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation Cleveland ,Ohio 44115,USA.

Hereditary defects of tooth dentin are classified into two main groups: dentin dysplasia (DD) (types I and II) and dentinogenesis imperfecta (DGI) (types I, II, and III). Type II DGI is one of the most common tooth defects with an autosomal dominant mode of inheritance, and one disease-causing gene, the dentin sialophosphoprotein (DSPP) gene, has been reported. To date, only 8 mutations have been reported in DSPP. In this study, we characterized a four-generation Chinese family with type II DGI that consists of 31 living family members, including 12 affected individuals. Linkage analysis with polymorphic marker D4S1534, 2.3 Mb from DSPP, showed complete linkage with the disease. Direct DNA sequence analysis identified a novel mutation (c.49CT, p.Pro17Ser) in exon 1 of the DSPP gene. The mutation was identified in all affected individuals, but not with normal family members and 100 controls. These results suggest that p.Pro17Ser causes DGI in the Chinese family. Interestingly, a different mutation, p.Pro17Thr, was previously identified at codon 17 in another Chinese family with type II DGI. All carriers with mutation p.Pro17Thr were associated with progressive high frequency hearing loss, however, carriers with p.Pro17Ser have normal hearing. This study identifies a novel mutation in the DSPP gene, and expands the spectrum of mutations that causes DGI.

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A new locus for autosomal dominant amelogenesis imperfecta. *G.A. Mendoza[1], T.J. Pemberton[1], M. Karaman[1], C. Gonzalez[1], V. Ninis[2], R. Scarel-Caminaga[3], J. Haartiala[1], K. Lee[4], S. Leal[4], M.L. Snead[5], J. Hacia[1], H. Allayee[1], S.R.P. Line[6], P.I. Patel[1,5].* 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) Department of Neurology, Baylor College of Medicine, Houston, TX; 3) Faculty of Odontology of Araraquara-UNESP, Brazil; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA; 6) Faculty of Odontology of Piracicaba-UNICAMP, Brazil.

Amelogenesis imperfecta (AI) is a collective term used to describe phenotypically diverse forms of defective tooth enamel development. Its prevalence varies between populations with it being common in Sweden where 1 in 700 are affected but less common in the United States where 1 in 14,000 are affected. AI has been reported to exhibit a variety of inheritance patterns and several loci have been identified that are associated with AI. To date, X-linked dominant AI has been associated with the amelogenin locus on Xq22.3, while autosomal dominant AI has been associated with the enamelin gene at 4q21 and the distal-less homeobox 3 gene at 17q21, and autosomal recessive AI has been associated with the kallikrein gene at 19q13.4. We have mapped a novel locus underlying AI to a region on the long arm of chromosome 8 by linkage analysis in a large Brazilian family segregating an autosomal dominant form of AI. Due to the large size of the region identified and limited meioses available, conventional fine mapping techniques to refine the critical interval were not possible. To identify positional candidate genes, we have performed microarray analysis of gene expression during molar tooth development in the mouse between post-natal days 1 through 10. These developmental stages encompass those where enamel is formed, thereby allowing the identification of candidate genes within our region of interest. Conventional fine mapping techniques can now be employed to analyze these candidate genes and identify the mutation causing AI in this family. (Supported by NIH grant DE014102).

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CDKL5 and the early onset seizure variant of Rett syndrome: clinical and molecular characterization. *C. Pescucci[1], F. Mari[1], S. Azimonti[2], R. Caselli[1], E. Scala[1], I. Longo[1], F. Ariani[1], V. Broccoli[3], F. Bolognese[2], I. Bertani[2], P. Balestri[4], M. Zappella[5], C. Kilstrup-Nielsen[2], N. Landsberger[2], A. Renieri[1].* 1) Medical Genetics, Siena, Italy; 2) Structural and Functional Biology, Busto Arsizio, Italy; 3) DIBIT, Milan, Italy; 4) Pediatrics, Siena, Italy; 5) Child Neuropsychiatry, Siena, Italy.

MECP2 is responsible for both classic and preserved speech variant of Rett syndrome (RTT). We report here the identification of mutations in the CDKL5 gene, encoding a putative kinase, in 4 RTT female patients: 2 early truncating mutations interrupting the catalytic domain (c.163_166del4; c.838_847del10) and 2 late truncating mutations (c.2635_2636del2, c.2343delG). The clinical course of patients is strikingly similar: they had seizures in the first months of life and subsequently developed recognizable RTT features. Two of them showed convulsions in the first days of life, the third at 1,5 months and the fourth at 6 months. Later on, they all had stereotypic hand activities, hand apraxia and the older patient (9 years) showed acquired microcephaly. RTT features were more evident in the older patients, in whom a clinical diagnosis of early onset seizure variant of RTT was promptly suspected. In the younger patient (2 years) the phenotype was less characteristic and clinical features were intermediate between early onset seizure variant of RTT and autism. We demonstrate that CDKL5 is a nuclear factor whose expression in the brain of developing mice significantly overlaps that of MeCP2. Moreover, we show in vitro and in vivo a direct interaction between MeCP2 and CDKL5. Functional characterization of CDKL5 showed that CDKL5 harbors an autophosphorylation activity, demonstrating that indeed it is a kinase. Furthermore we were able to demonstrate that CDKL5 mediates MeCP2 phosphorylation. In conclusion, our results contribute to the clarification of the phenotype associated with CDKL5 mutations and trace out a molecular link between MeCP2 and CDKL5. Further studies are necessary to firmly establish whether MeCP2 is the main target of CDKL5 in vivo and whether the biological significance of the interaction is limited to phosphorylation.

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Identification and functional analysis of the mouse homologue of the D4Z4 repeat that is mutated in facioscapulohumeral muscular dystrophy. *J.E. Hewitt, L. Mitchell, P.J. Scotting, J. Clapp.* Inst Genetics, Queens Med Ctr, Univ Nottingham, Nottingham, United Kingdom.

Facioscapulohumeral muscular dystrophy (FSHD) is caused by a unique genetic rearrangement close to the telomere of human chromosome 4q. The disease is causally associated with deletions within a tandem DNA repeat (D4Z4). Initially, because of homeobox sequences within D4Z4, it was thought that the mutation might alter a protein product encoded by the repeat. However, although cDNAs and RT-PCR products containing closely related sequences have been identified, none of these originate from D4Z4. An alternative mechanism is that the deletion alters the local chromatin environment, resulting in a position effect on one or more nearby genes. Although changes in 4q35 gene expression levels in FSHD muscle have been reported, these findings remain controversial. It is apparent that the function of D4Z4 and its role in FSHD remains to be defined. In light of this, we have returned to study the evolution of D4Z4 as this may shed further light on its function. Although DNA hybridization techniques have previously identified D4Z4 homologues only in primates, here we show that the mouse genome also contains sequences homologous to D4Z4. These are arranged in a large (100-200kb) tandem array, with each 5kb repeat unit having an open reading frame containing two homeobox sequences. The rat genome contains an orthologous array at the equivalent genome location. Phylogenetic analysis indicates that human and mouse D4Z4 homeodomains are more closely related to each other than to other homeodomains. By RT-PCR and in situ hybridisation we have shown that the mouse array (mD4Z4) is transcribed in a wide range of embryonic and adult tissues, including skeletal muscle. Transfection of epitope-tagged mD4Z4 into C2C12 cells shows the protein to localise to the nucleus. This is the first time that any D4Z4 homologous sequence, apart from very closely related primate species, has been identified and suggests that the hypothesis that the human D4Z4 repeat encodes a protein should be revisited.

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Identification of Novel Mutations in the SOX2 Gene in Patients With Microphthalmia/Anophthalmia. *T. Young[1,2], J. Zhou[1,2], T. Bardakjian[3], F. Kherani[1], J. Katowitz[1], N. Hughes[2], L. Schimmenti[4], A. Schneider[3].* 1) Division of Ophthalmology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Genetics, Albert Einstein Medical Center, Philadelphia PA; 4) Division of Genetics, University of Minnesota, Minneapolis MN.

The SOX2 gene, located at chromosome 3q27-q28, encodes a Sry-related transcription factor containing an HMG (high-mobility-group) DNA-binding domain and plays a key role in cell fate determination. Mutations of the SOX2 gene have been reported in 13 sporadic cases of patients with anophthalmia or microphthalmia. In this study, a set of 31 anophthalmic/microphthalmic patient DNA samples were screened for sequence changes by gel shifts in the SOX2 gene initially by conformation-sensitive gel electrophoresis. Abnormal amplicon patterns were subsequently screened by direct sequencing. Two novel heterozygous mutations were identified in two patient samples. The first sequence alteration was a single nucleotide mutation G737T located in the HMG DNA binding domain. The mutation changes the codon from glutamic acid (GAG) to a stop codon (UAG). The predicted product would produce a truncated protein and disable the DNA binding function. The second mutation was a single nucleotide deletion-C976del in the activation domain, resulting in a frameshift with premature termination of the coding sequence. However, the truncated protein would most likely be destroyed by non-sense mediated decay. Both mutations strongly suggest loss of function. In addition, 3 single nucleotide polymorphisms (SNPs), G1603A, C2050A, and G2138A, were identified in the 3-untranslated region in 3 other patients. The C2050A SNP (rs11915160) has been reported previously in normal individuals. The relationship between the remaining two SNPs and anophthalmia/microphthalmia is indeterminate. These results support the role of SOX2 in ocular development, and provide evidence that SOX2 loss of function mutations result in severe eye malformations.

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Genetic analysis of congenital generalized lipodystrophy type 2 in Chinese. *YJ. Lee, CY. Huang, SP. Lin, CL. Lin, HF. Liu, CD. Tsai, HJ. Li.* Dept Pediatrics & Medical Res, Mackay Memorial Hosp, Taipei, Taiwan.

Congenital generalized lipodystrophy (CGL) was originally described by Berardinelli and Seip. It is a rare autosomal recessive disorder and characterized by a near total lack of adipose tissue from early infancy, acanthosis nigricans with severe insulin resistance, hypertriglyceridemia, and hepatomegaly with fatty change. Early childhood is marked by accelerated linear growth, an advanced bone age, and a voracious appetite. Later in childhood, marked acanthosis nigricans usually develops in the neck, axilla, groin, and trunk. Clinically it is classified into two types. The patients with CGL type 2 often have mental retardation, whereas those with CGL type 1 rarely show intellectual impairment. The mutations of the AGPAT2 and Seipin genes have been detected in CGL type 1 and type 2, respectively. **Patients and healthy adult controls:** Three pediatric patients with typical clinical manifestations of CGL and mental retardation and their families as well as 50 normal adults were recruited. The parents denied consanguinity. The study was approved by the IRB of the hospital and all subjects gave informed consents. **Methods:** Genomic DNA was extracted by the standard method. Primers to amplify the exons of the Seipin gene including exon-intron junctions were designed by using PrimerQuest. The entire coding regions of the gene were sequenced and homology search was done by using BLAST. **Results:** Two mutations were detected. Mutation nt890insCCG was in exon 5 and caused a change of amino acid and an insertion of an additional amino acid (Glu182Asp;182insArg). The other mutation nt1127insG was in exon 7 and caused a change of amino acid and a frameshift which resulted in a premature termination of translation and a truncated protein (Ile262His fsX273). The patients were homozygous and the parents were heterozygous. None of the mutations were detected in normal controls. We searched Human Gene Mutation Database and NCBI and confirmed that the two mutations were novel. **Conclusion:** Mutations of the Seipin gene in Chinese are different from those reported in Caucasians and Japanese. More extensive analysis in a larger dataset is necessary to detect similar mutations.

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Identification of novel Edar and Edaradd gene mutations and their impact on NF-kB activation in anhidrotic ectodermal dysplasia. *C. Cluzeau[1], S. Hadj-rabia[2], H. Turki[3], C. Bodemer[2], JP. Bonnefont[1], A. Munnich[1], A. Smahi[1].*
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Anhidrotic ectodermal dysplasia (EDA) is a disorder of ectodermal differentiation characterized by sparse hair, abnormal or missing teeth and inability to sweat. X-linked EDA is the most common form, caused by mutations in ectodysplasin, a member of the TNF family. Autosomal dominant and recessive forms of EDA, indistinguishable from the X-linked form, have been also described. Two different disease genes, EDA-2 or EDA-3, have been identified. Mutations in EDA-3 encoding EDAR, a TNF receptor, result in either dominant or recessive forms. In addition, mutations in a recently identified gene, EDA-2 encoding EDARADD (for Edar-Associated Death Domain) have also been shown to cause recessive EDA. We performed genetic analysis in two unrelated consanguineous families with autosomal recessive EDA. In family 1 originating from Tunisia, we found linkage at the EDA-2 locus and identified a deletion of exon 4 in EDA-2, which likely results from mis-appariement of repeated sequences flanking exon 4. The resulted protein is about 50 amino-acid long and lacks the death domain (DD) which is involved in interaction between Edar and Edaradd via their respective DD, and in homodimerization of Edaradd molecules. We showed that Edaradd mutant lacking the DD is unable to activate an NF-kB-driven luciferase reporter construct. In family 2, originating from Koweit, linkage was found at the EDA-3 locus. Screening of 12 exons of the gene revealed a mutation (1207, C to T) which changed threonine into a methionine at amino acid 403 of the Edar protein. We demonstrated that the T403M mutation observed in Edar receptor DD severely impairs NF-kB activation. The exon 4 deletion in the Edaradd gene and the T403M Edar mutation impaired NF-kB activation and probably resulted in abnormal differentiation of skin appendages responsible for EDA phenotypes in our families.

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Caspase-1-mediated cleavage of pyrin, the familial Mediterranean fever protein, activates NF-B. *J. Chae[1], K. Richard[1], G. Wood[1], H. Jaffe[2], D. Gumucio[3], N. Shoham[1], D. Kastner[1].* 1) Genetics and Genomics Branch, NIAMS/NIH, Bethesda, MD; 2) Protein/Peptide Sequencing Facility, NINDS/NIH, Bethesda, MD; 3) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

Autoinflammatory diseases such as familial Mediterranean fever (FMF) represent inherited disorders of innate immunity, and are characterized by seemingly unprovoked fevers and serosal, synovial, or cutaneous inflammation. FMF is caused by recessive mutations in MEFV, encoding a 781-amino acid (aa) protein denoted pyrin. Carrier frequencies as high as 1:3 have been observed in the Middle East. The N-terminal 92 aa of pyrin define a death domain-like motif, PYD (PAAD, DAPIN), that mediates cognate interactions with the adaptor protein ASC, thereby regulating caspase-1 and IL-1 activation. However, most FMF mutations are in the C-terminal B30.2 domain, the function of which is unknown. . Here we demonstrate that pyrin can regulate caspase-1 by direct interaction through the catalytic domains of caspase-1 and B30.2 domain of pyrin, where most of the major FMF mutations are present. The binding affinity of caspase-1 is decreased with the three major pyrin mutants in comparison with normal pyrin. Moreover, caspase-1 cleaves pyrin at Asp330, producing an N-terminal fragment that translocates to the nucleus. The N-terminal fragment also interacts with IB- which is associated with degradation of IB- and NF-B activation. B30.2 mutants undergo increased caspase-1-mediated cleavage, relative to wild type. Moreover, absolute and relative quantities of cleaved pyrin are substantially increased in leukocytes from FMF patients compared with healthy controls, and the relative amount of cleaved N-terminal fragment is correlated with the degradation of IB-. These data identify a new pyrin/caspase-1 pathway for NF-B activation, and suggest a molecular basis for selection of pyrin mutants in man.

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Genetic susceptibility to neonatal-onset multisystem inflammatory disease (NOMID/CINCA): novel *CIAS1* mutations and genomic strategies to resolve locus heterogeneity. *I. Aksentijevich[1], J. Balow Jr.[1], J. Jones[2], N. Dailey[2], S. Canna[2], E.F. Remmers[1], R. Goldbach-Mansky[2], D.L. Kastner[1].* 1) Genetics & Genomics Branch, NIH/NIAMS, Bethesda, MD; 2) Office of Clinical Director, NIAMS/NIH, Bethesda, MD.

Neonatal-onset multisystem inflammatory disease (NOMID/CINCA) is a rare autoinflammatory disease characterized by fever, chronic meningitis, uveitis, sensorineural hearing loss, urticarial rash, and a characteristic deforming arthropathy. NOMID is caused by missense mutations in *CIAS1* (also known as NALP3, PYPAF1) that encodes cryopyrin. NOMID is allelic with Muckle-Wells syndrome (MWS) and familial cold autoinflammatory syndrome (FCAS), two dominantly inherited disorders with some similarities to NOMID. Currently, over 35 disease-associated mutations have been found and all are clustered in the NACHT domain of the protein. We previously reported 6 mutations in 13 NOMID cases. Subsequently, we analyzed 23 new patients and collected PBMC from 17 patients for gene expression studies. In 6 patients we identified new missense changes: V262A, L264F, V351L, G326E, F443L, and F523C, while 8 patients carried previously described mutations. Thus, including our previous report we evaluated 36 NOMID patients and we found mutations in 20/36. There was no clinical difference between mutation positive and negative patients and they both responded dramatically to anakinra treatment. We used Affymetrix U133A microarrays to identify disease-specific gene expression profiles in PBMC of 17 NOMID patients at baseline and during their treatment with the IL-1RN (anakinra). 14 samples were paired; therefore we carried out both paired and unpaired t-test analysis. Among the genes that are at least 2 fold up-regulated in patients prior to treatment at a significance level of p 0.01 are several cytokines, cytokine receptors (including IL-1RN), genes that are involved in the immune response, regulation of apoptosis, and cell adhesion. We identified *CIAS1* as 2.6 fold up-regulated in NOMID patients during inflammation. Through these studies we hope to uncover novel candidate genes for the screening of *CIAS1* mutation negative patients.

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Genetic mapping of autosomal dominant ocular coloboma. *S. Duan, L. Wang, Y. Zhao, Z. Yang, K. Zhang.*
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Purpose: Ocular coloboma is a congenital abnormality caused by defective closure of the embryonic fissure of the optic cup. The defect is typically located in the lower part of the eye. Iris, ciliary body, choroids, retina and optic nerve can be affected. The purpose of this study is to identify a locus responsible for ocular coloboma in a Chinese family. Methods: A large Chinese family with an autosomal dominant form of ocular coloboma was investigated. No other systemic malformations were observed. Genotyping with STR markers linked to known loci/genes associated ocular coloboma was performed. Candidate loci included VAX2,SHH,PAX2,VAX1,PAX6,CHX10 on the regions of chromosomes 2, 7, 10, 11, 12, and 14 respectively. Results: There were nine affected individuals spanning three generations. Affected individuals presented with iris, retina, choroids, and optic nerve coloboma. Central vision was affected in two patients. No other systemic malformations were observed. Linkage to candidate loci VAX2,SHH,PAX2,VAX1,PAX6,CHX10 were excluded. Conclusions: Our results suggested a novel locus related to ocular coloboma. Genome wide scan is in progress to map this disease gene. Idnetification of a gene for ocular coloboma may provide valuable insight into ocular development.

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Genetic study of a large Chinese amyotrophic lateral sclerosis family. *Y.Q. Song[1, 2], W.L. Ho[3], H.H. Kwok[3], C.Y. Chu[3], Y. Li[1], S.L. Ho[3].* 1) Dept Biochemisty, Univ Hong Kong, Hong Kong, China; 2) Genome Research Centrre, Univ Hong Kong, Hong Kong, China; 3) Dept Medicin,Univ Hong Kong, Hong Kong, China.

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder arising from the progressive death of motor neurons in the cortex, brain stem, and spinal cord, and resulting in muscle weakness, wasting, and respiratory paralysis and the life expectancy is about 3 to 5 years after symptom-onset. About 10 % of cases are familial (FALS), but there are several subtypes with different modes of inheritance and different clinical features. We have recently identified a large multigenerational Chinese family in Hong Kong with autosomal dominant ALS. This family displayed typical ALS, with onset in the 30s to 40s of mixed upper and lower motor neuron degeneration, bulbar dysfunction and rapid progression to respiratory failure. However, some members had a more slowly progressive, predominantly lower motor neuron lesion in the lower limbs, with relatively sparing of the upper limbs. So far, we have documented over 130 members and collected 70 DNA in this four-generation family. We have screened all five exons of the SOD1 gene for mutations using both single-strand conformational polymorphism (SSCP) and direct nucleotide sequencing methods. A mutation of T/C transition, at codon 151 in exon 5 was identified. This mutation results in the substitution of an isoleucine for a theronine, and is thought to affect the formation of dimers in SOD1. Moreover, As the different branches showed different severity of the phenotypes, a modifier or modifiers may exist in this family. We are now sequencing the known MND modifier genes (ie, VEGF and CTNF). At the same time, we have done a genome wide screen using the Illumina 5,900 SNP panel. The data is being analyzed and we will report our results during the meeting.

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Atypical Presentations of Hereditary Lymphedema Type I Associated with Mutations in *Vascular Endothelial Growth Factor Receptor 3*. *A. Ghalamkarpour[1], A. Raas-Rothschild[2], R. Spiegel[3], A. Utkus[4], S.A. Shalev[3], L.M. Boon[1,5], M. Vikkula[1].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute and University of Louvain Medical School, Brussels, Belgium; 2) Department of Human Genetics, Hadassah Hebrew University Hospital, Jerusalem, Israel; 3) Genetics Institute, HaEmek Medical Center, Afula, Israel; 4) Department of Human and Medical Genetics, Vilnius University, Vilnius, Lithuania; 5) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires Saint-Luc, Brussels, Belgium.

Genetic studies have identified mutations in the *vascular endothelial growth factor receptor 3* gene, *VEGFR3/FLT4*, in some families with hereditary lymphedema type I (Milroy disease; MIM 153100). Individuals carrying a *VEGFR3* mutation exhibit congenital edema in the lower limbs, usually bilaterally and below the knees, sometimes associated with cellulitis, prominent veins, papillomatosis, upturned toenails, and hydrocele. In this study, we present clinical variability in four families in which we identified a *VEGFR3* mutation. The clinical findings in two families varied from spontaneous resorption of the edema to elephantiasis. The index case in the third family was discovered to have polyhydramnios, massive bilateral hydrothorax, skin edema, scalp edema and minimal ascites at 33 weeks of gestations. The index case in the fourth family presented prenatally with bilateral leg edema, bilateral hydrothorax and lung hypoplasia at 22 weeks of gestation. The association of prenatal pleural effusion and hydrops fetalis with a *VEGFR3* mutation has not been described earlier. Therefore, mutations in *VEGFR3*, similar to the mutations in the two other lymphedema associated genes, i.e. *FOXC2* and *SOX18*, can cause hydrops fetalis. These results are consistent with animal models with a *Vegfr3* mutation (chy mouse) presenting edema elsewhere than the limbs (chylous ascites). We conclude that the phenotypic spectrum of hereditary lymphedema type I associated with a *VEGFR3* mutation is much wider than previously appreciated. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be).

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Molecular Chaperones in Gaucher disease. *D. Urban[1, 2], O. Goker-Alpan[1, 2], B.K. Stubblefield[1, 2], E. Sidransky[1, 2].*
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Protein folding is crucial not only for generating biologically active proteins, but also for maintaining many vital intra-cellular processes, such as trafficking. It depends on many factors such as pH, temperature and protein quality control systems within the cell. During cellular stress, molecular chaperones are activated to ensure folding and prevent protein aggregation. In Gaucher disease, the inherited deficiency of the lysosomal enzyme glucocerebrosidase, most mutations are missense, and are expected to result in the synthesis of an unstable protein. We investigated protein folding and the effects of quality control systems in fibroblasts from patients with Gaucher disease. As mutant proteins refold at lower temperatures without the need of chaperones, cell lines with different Gaucher genotypes were grown at 37C and 30C to test the stability of mutant glucocerebrosidase. In most, mutant glucocerebrosidase was misrouted to early endosomes at 37C. At 30 C, trafficking appeared to be corrected and the residual enzyme activity and steady-state protein levels were increased, especially in cell lines carrying mutation L444P. These findings suggest that mutant glucocerebrosidase is misfolded. The molecular chaperones HSP-90, 70 and 60 were examined in fibroblasts from patients with Gaucher disease, and the steady state HSP levels were followed at the different temperatures. HSP90, a chaperone that prevents aggregation, was decreased in most of the cell lines. The mitochondrial chaperone HSP-60 also showed some variation, but HSP-70 levels remained constant. Since HSP-90 deficiency results in proteosomal dysfunction, mutations in glucocerebrosidase may lead to both aggregation and decreased degradation and clearance by interfering with the ubiquitin-proteosome system. Molecular chaperones may act as genetic modifiers in Gaucher disease and contribute to atypical phenotypes, such as parkinsonism, that are related to protein misfolding and aggregation.

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Fabry disease: Identification of 48 novel -galactosidase A mutations and 3-D structural analysis of 29 missense mutations. *J. Shabbeer[1], M. Yasuda[1], S.D. Benson[2], R.J. Desnick[1].* 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Dept Chemistry, Oklahoma State University, Stillwater, OK.

Fabry disease, an X-linked recessive inborn error of glycosphingolipid catabolism, results from the deficient activity of the lysosomal exoglycohydrolase, -galactosidase A (EC 3.2.1.22; -Gal A). The molecular lesions in the -Gal A gene causing the classic phenotype of Fabry disease in 64 unrelated families were determined. In 47 families, 48 new mutations were identified, including: 29 missense (N34K, T41I, D93V, R112S, L166G, G171D, M187T, S201Y, S201F, D234E, W236R, D264Y, M267R, V269M, G271S, G271V, S276G, Q283P, A285P, A285D, M290I, P293T, Q312H, Q321R, G328V, E338K, A348P, E358A, Q386P), eight nonsense (C56X, E79X, K127X, Y151X, Y173X, L177X, Q306X, E338X), five splicing defects (IVS4-1G>A, IVS5-2A>G, IVS5+3A>G, IVS5+4A>G, IVS6-1G>C), four small deletions (18delA, 457delGAC, 567delG, 1096delACCAT), one 3.1 kb Alu-Alu deletion which included exon 2, and one complex mutation (K374R, 1124delGAG). In 18 families, 17 previously reported mutations were identified, with R112C occurring in two families. Two classically affected males had -Gal A alleles with two mutations, D264Y/V269M and A143T/Q312H. Transient expression of the individual mutations revealed that D264Y and Q312H were localized in the ER and had no detectable or markedly reduced activity, whereas V269M and A143T were localized in lysosomes and had ~10% and ~35% of expressed wild-type activity, respectively. Structural analyses based on the enzymes 3-D structure predicted the effect of the 29 novel missense mutations on the mutant proteins structure. Of note, three (~10%) were predicted not to alter the enzyme's structure, but were disease causing, indicating the limitation of structure function predictions. These studies further define the molecular heterogeneity of the -Gal A mutations in classical Fabry disease, and provide insights into the structural enzyme alterations that cause the classic phenotype.

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Nonsense and frameshift ESCO2 mutations in Roberts syndrome and SC phocomelia. *B. Schuele[1], K. Johnston[2], U. Francke[1].* 1) Department of Genetics, Stanford University, Stanford CA; 2) Department of Genetics, Kaiser Permanente, San Francisco CA.

Roberts syndrome (RBS, MIM# 268300) is a rare AR disorder characterized by craniofacial anomalies, microcephaly, mental deficiency, profound growth deficiency, and tetraphocomelia. SC phocomelia syndrome (MIM# 269000) has a milder phenotype with a lesser degree of limb reduction. Since repulsion of heterochromatin regions (PCD) is characteristic for both disorders and complementation studies placed both into the same group, it is likely that the disorders are allelic. Recently, mutations in the ESCO2 gene on 8q21.1 have been reported in 15 families with RBS (Vega et al., Nat Genet 37:468, 2005). The yeast homolog Eco1 is required for the establishment of sister chromatid cohesion during S phase. We have studied 5 affected individuals from 4 families for mutations in ESCO2. PCD was documented in all affected individuals. Two of the families had previously been reported as SC phocomelia with a milder phenotype and survival into adulthood. In the other two families, severe limb and craniofacial abnormalities, detected by fetal ultrasound, led to a diagnosis of RBS and termination of pregnancy. PCR amplicons of all 10 coding exons, including the intron-exon boundaries, were sequenced bi-directionally. We identified six mutations in three compound heterozygotes in the non-consanguineous families and one homozygous deletion in a consanguineous family: two nonsense mutations Q202X (c.604 C>T) and W423X (c.1269 G>A), three frameshift mutations (c.752delA, c.751_752insA, c.307_311del AGAAA), and one mutation probably involved in splicing (c.1132 -7A>G). One of the frameshift mutations (c.752delA) was identified in two unrelated families. All mutations we detected are different from the 8 previously reported ones. These data provide proof that RBS and SC phocomelia are caused by mutations in the same gene. As inactivating nonsense and frameshift mutations were identified in mildly as well as severely affected individuals, no phenotype-genotype correlation could be made. To discover the modifying mechanisms which lead to the phenotypic variability will be an important future task.

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Cryptic genomic imbalances as a cause of syndromic X-linked mental reatardation. *A.M. Vianna-Morgante[1], J. Knijnenburg[2], P.A. Otto[1], A.C. Krepischi-Santos[1], R.M.P. Nascimento[1], C. Rosenberg[1,2].* 1) Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil; 2) Laboratory of Cytochemistry and Cytometry, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.

We used array-CGH with approximately 3500 large insert clones spaced at ~1 Mb intervals through the genome, constructed at the Leiden University Medical Center, to investigate DNA copy number changes in three unrelated mentally impaired Brazilian males, the probands from syndromic X-linked recessive mental retardation families (S-XLMR). Their karyotypes were normal and they tested negative for fragile X syndrome. The normal mothers of affected individuals had completely skewed X-inactivation as demonstrated by the methylation status of the AR locus. Two of these X-linked cases showed DNA copy number imbalances, respectively a duplication of one BAC clone at Xq28 (confirmed by MLPA in the propositus and in an affected maternal cousin), and a partial deletion of a single clone at Xp11.23 (confirmed by FISH in an obligate heterozygote). Since these inherited imbalances had not been previously reported or detected by us in more than 100 X-chromosome control observations, they were considered causative of S-XLMR. The imbalanced segments contained several genes. One of these genes may have a major effect, but the abnormal phenotypes may well represent contiguous gene disorders. We show here that genomic rearrangements producing DNA copy imbalances on the X-chromosome may segregate in families in a pattern similar to recessive gene mutations.

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Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates eyelid conditioning in a manner which phenocopies human Fragile X syndrome. *B. Oostra[1], S. Koekkoek[2], K. Yamaguchi[3], R. Maex[4], M. Vellema[2], F. van der Werf[2], E. De Schutter[3], C. Bakker[1], R. Willemsen[1], D. Nelson[5], E. Mientjes[1], M. Ito[3], C. De Zeeuw[2].* 1) Dept Clin Genet, ErasmusMC, Rotterdam, Netherlands; 2) Dept Neuroscience, ErasmusMC, Rotterdam, Netherlands; 3) Lab for Memory and Learning and Lab for Behav Genet, Brain Sci Institute, RIKEN, Japan; 4) Lab Theor Neurobiol, Univ Antwerp, Belgium; 5) Dept of Mol and Hum Genet, Baylor College of Medicine, Houston, Texas.

Absence of functional FMRP causes fragile X syndrome. Before we have generated an *Fmr1* knockout mouse, in which FMRP is absent, and which shows behavioral and cognitive abnormalities comparable to the symptoms found in fragile X patients and several of them can be linked to a dysfunction of a particular brain region. Abnormalities in synaptic processes in the cerebral cortex and hippocampus contribute to cognitive deficits in fragile X patients. So far the potential roles of cerebellar deficits have not been investigated. Metabotropic GluR1-dependent LTD, which appears to require rapid translation of mRNA, can also be induced at the parallel fiber to Purkinje cell synapse in the cerebellum. To investigate the possibility that cerebellar deficits contribute to fragile X syndrome we tested cerebellar learning capabilities of global and Purkinje cell specific Fmr1 knockout mice as well as of fragile X patients using classical eyeblink conditioning procedures. We demonstrate that both global and Purkinje cell specific knockouts of Fmr1 show deficits in classical delay eyeblink conditioning in that the percentage of conditioned responses as well their peak amplitude and peak velocity are reduced. Purkinje cells of these mice show elongated spines and enhanced LTD induction at the parallel fiber synapses that innervate these spines. Moreover, fragile X patients display the same cerebellar deficits in eyeblink conditioning as the mutant mice. These data indicate that a lack of FMRP leads to cerebellar deficits at both the cellular and behavioral level and they raise the possibility that cerebellar dysfunctions can contribute to motor learning deficits in fragile X patients.

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PLP2-deficient mice have increased sensitivity to ER stress and apoptosis. *L. Zhang[1], D. Valle[1,2].* 1) Inst. of Genet. Med., Johns Hopkins Univ; 2) Howard Hughes Med. Inst., Baltimore, MD.

Mental retardation (MR) is a common cause of handicap in children and young adults. Previously, we identified a functional promoter SNP (-113C>A) in the proteolipid protein 2 (PLP2) gene which abolishes the core-binding site of a neuronal transcription factor, ELK1, results in significant reduction (> 4-6 fold) of PLP2 mRNA and protein and is over-represented in males with X-linked MR, particularly those with mild cognitive impairment (relative risk = 2.5). To understand PLP2 function, we generated Plp2 null mice by gene targeting. PLP2 -/- and -/Y mice have normal viability and fertility and have no gross anatomical abnormalities. Since PLP2 has been shown to interact with BAP31, an ER protein involved in apoptosis, we examined programmed cell death in the Plp2 -/- and -/Y cells by culturing them in agents that stimulate specific apoptotic pathways: VP16 (intrinsic pathway), Fas ligand (extrinsic pathway), thapsigargin (ER stress), and tunicamycin (ER stress). Plp2-deficient cells are indistinguishable from control cells in their response to stimulation of the intrinsic and extrinsic pathways but showed increased sensitivity to ER stress. Thapsigargin (2uM) killed 3.5 fold more Plp2-deficient cells (54%) than the controls (12%); tunicamycin (2uM) killed 2.7 fold more Plp2-deficient cells (59%) than the controls (16%). In agreement with these results, stimulating ER stress in Plp2-deficient cells resulting in a higher level of the apoptotic markers (caspase 3 and its nuclear target, PARP; caspase 12, an ER stress-specific caspase and GRP78, an ER chaperone that is elevated during ER stress). Interestingly, we also found an elevated level of basal ER stress (increased caspase 12 and GRP78) in the Plp2-deficient cells grown under standard conditions. These results suggest that PLP2 deficiency is associated with an increased basal level of ER stress and an exaggerated apoptotic response to agents that induce apoptosis. We speculate that individuals with the PLP2 -113C>A SNP may also have increased sensitivity to ER stress, and that PLP2 could be a modifier gene for missense mutations in proteins that traverse the ER.

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Inactivation of the *Drosophila* Neurotrypsin homolog results in long-term memory defect. *G. Didelot[1], F. Molinari[2], A. Munnich[2], T. Preat[1], L. Colleaux[2].* 1) DEPSN, CNRS, Gif-sur-Yvette, France; 2) INSERM U393, Hôpital Necker-Enfants Malades, Paris, France.

Neurotrypsin is a neuronal serine protease predominantly expressed in neurons of the cerebral cortex, the hippocampus and the amygdala. Truncating mutation of the human gene (PRSS12) causes autosomal recessive nonsyndromic mental retardation and several studies in mice suggested that this protein may be a regulator of adaptative synaptic functions. However, the precise function of neurotrypsin protein remains elusive.

To gain insight into the pathophysiological basis of cognitive impairment in patients with neurotrypsin mutation, we studied the Drosophila *tequila* gene, the drosophila ortholog of Neurotrypsin. Drosophila is indeed an attractive system to unravel molecular and cellular mechanism involved in associative learning and memory as it is easily amenable to genetics, biochemical, molecular and behavioural analyses. We first showed, by immunohistochemistry, that Tequila protein is preferentially expressed in the mushroom bodies (MBs), a prominent bilateral structure of the insect brain essential for learning and memory . We then constructed a *tequila* knock-down Drosophila line using RNA interference and designed to specifically inactivate *tequila* expression in the MBs.

Here we report results from the analysis of *tequila* "knock-down" flies and show that mushroom bodies organogenesis, learning and short-term memory (STM) were all normal. By contrast, long-term memory (LTM) is clearly defected in *tequila* "knock-down" flies compared to controls. Moreover, we observed that, in wild-type flies, LTM conditioning induces a transient increase of *tequila* expression.

These data strongly support that Drosophila is a suitable model for the studying the pathophysiological mechanism resulting from neurotrypsin mutation. In addition our results suggest that tequila, and therefore neurotrypsin, is likely involved in synaptic plasticity and that this protease may be a key player in the pathway underlying LTM formation.

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Conditional knockout mouse model for the *Fxr2* gene. *S. Pataskar[1], B.A. Oostra[2], D. Nelson[1].* 1) Molecular & Human Genetics,Baylor College Of Medicine, Houston, TX, USA; 2) CBG Department Of Clinical Genetics, Erasmus University, Rotterdam,The Netherlands.

Fragile X syndrome is a common form of mental retardation caused by the absence of the *Fmr1* protein, FMRP (Fragile X Mental Retardation Protein). Two paralogs of FMRP have been identified , FXR1P and FXR2P (Fragile X Related Protein). All three proteins share highly conserved RNA binding domains, high sequence similarity and show overlap in their tissue distribution. FXR2P shows high expression in brain and testes similar to FMR1P. It is likely that FXR1P and FXR2P provide functional redundancy for FMR1P. This redundancy masks the phenotypic consequences of loss fucntion mutations in humans and mice. To unravel the biological mechanisms involved in the normal and abnormal functioning of FXR2P and FMR1P, we are developing knockout mice lacking each of the genes, and combinations, including mutations that can be conditionally expressed via the *Cre/loxP* system. To make a conditional mutation in mouse *Fxr2* gene, we have elected to construct a targeted allele with *loxP* sites flanking exon 7. This mutation should provide the capability to conditionally ablate the gene using tissue specific or time dependent expression of *Cre* recombinase. The mutant mice will be analysed by biochemical, histological and behavioral methods in order to determine the consequences of absence of *Fxr2* gene in specific tissues. A targeting construct has been introduced into mouse ES cells and homologous recombinations have been identified. *Cre* recombinase has been introduced and ES cell lines are being characterised for correct recombination. A conditional mutation in *Fxr2* will allow us to investigate tissue specific or developmental timing effect of gene ablation. Mutations in *Fxr2* gene especially in combination with *Fmr1* mutation should help to resolve the question of functional redundancy.

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Identifying Proteins that modify the neurodegenerative phenotype of the Fragile X CGG90 repeats in Drosophila melanogaster. *O. Sofola, D. Nelson, J. Botas.* Human Genetics, Baylor College of Medicine, Houston, TX.

Fragile X Syndrome is the most common form of hereditary mental retardation. It is caused by a large expansion of the CGG trinucleotide repeat (>200 repeats) in the 5 untranslated region of FMR1 that leads to transcriptional silencing of the gene. Individuals with CGG repeat expansions between 60 and 200 are referred to as premutation carriers. These premutation carriers are able to transcribe FMR1 and hence are phenotypically normal with respect to the features of Fragile X syndrome. However, a neurodegenerative disorder has recently been described in premutation carriers. A transgenic fly model expressing the 5 UTR of the human FMR1 gene with CGG90 repeats was designed by Jin and colleagues to examine whether the premutation length repeat could cause neurodegeneration. The repeats are transcribed into RNA but are not translated into protein; flies expressing these CGG repeats display a disorganized eye phenotype, a characteristic marker of neurodegeneration in flies. Furthermore, the eye phenotype could be suppressed by over expressing hsp70, a chaperone involved in protein folding. These results suggested that transcription of the CGG90 repeats lead to an RNA mediated neurodegenerative disease, possibly via influencing RNA binding proteins. We are in the process of identifying potential RNA binding proteins using a genetic screen in CGG90 flies. This effort to define modifiers is being carried out using a set of candidate binding proteins and with an unbiased screen using ~450 fly strains deleted in different parts of their genome. The screen involved mating the CGG90 transgenic flies with other mutant flies that expressed either a reduced or an increased level of different proteins. The progeny were examined for potential suppression or enhancement of the disorganized eye phenotype seen in control CGG90 transgenic flies. Three deficiency stocks (7713, 7723, 7772) appear to suppress and two (7707, 7544) appear to enhance the CGG90 eye phenotype. At the moment, we are currently trying to narrow down which gene within the deleted region may be responsible for the modification of the eye phenotype and utilize other assays to confirm these modifiers.

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Role of Dp71 in the stability of the dystrophin associated proteins complex in the nucleus of PC12 cells. *M. Villarreal Silva[1], R. Rodriguez Munoz[1], F. García Sierra[2], B. Cisneros[1].* 1) Genetics & Molecular Biology Department; 2) Cellular Biology Department, CINVESTAV-IPN, D.F, Mexico.

Dp71, the major product of DMD gene in brain, is the smallest isoforms of the DMD gene. Due to the presence of mental retardation in a third of the DMD patients, it is believed that Dp71 has an important neuronal function. We have reported that aminoacids codified by exon 78 determine the nuclear localization of Dp71d isoform in PC12 and HeLa cell lines. In addition, we have observed the existence of some dystrophin-associated proteins in the nuclei of HeLa cells, and their interaction with the nuclear matrix proteins lamin and actin, suggesting the presence of a new DAPC in this organelle. Based on the preceding information, we have adopted the PC12 cell line as neuronal model for studying the nuclear function of Dp71, due to their ability to differentiate into sympathetic-like neurons. In this study, we have analyzed the effect of Dp71 levels in the DAPs distribution in the nuclear matrix of a wild type clone and Dp71-antisense clones (ASC). Our results show that decreased levels of Dp71 affect the levels of -dystroglycan and - and -dystrobrevins in the nucleus and nuclear matrix of ASC. Surprisingly, lamin B1 is also affected by Dp71 deficiency. Our results suggest that Dp71 has a role in the nuclear organization of PC12 cells.

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Increased glutamic acid decarboxylase expression in the fragile X mouse brain is independent of Fmrp expression. *W.T. Brown[1], A. El Idrissi[2], D. Ziemnicka[1], X.H. Ding[1], C. Dobkin[1].* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Center Developmental Neuroscience, College of Staten Island/CUNY, Staten Island, NY.

Increased seizure susceptibility is a feature of the mouse model for fragile X that has parallels in the hyperarousal and prevalence of seizures in the fragile X syndrome. Our investigation of the basis for the increased seizure susceptibility of the fragile X mouse indicated a reduction in GABAA receptor expression (El Idrissi et al. Decreased GABAA receptor expression in the seizure-prone fragile X mouse. Neuroscience Letters, 2005; 377:141-146). This receptor is a major component of the inhibitory (GABAergic) system and its reduced expression probably contributes to the increased seizure susceptibility observed in this mouse model for fragile X. We also found a potentially related alteration of the GABAergic system--increased expression of glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis. To investigate the intracellular dependence of GAD expression on Fmrp expression, we examined heterozygous female mice that expressed Fmrp and enhanced green fluorescent protein (EGFP) from one X chromosome and no Fmrp from the other X. Due to X inactivation only one chromosome is expressed in any somatic cell. Thus, these females were mosaic for Fmrp expression and cells expressing Fmrp were marked by EGFP. Analysis of the brains of these mice by immunostaining and confocal microscopy indicated that GAD expression was essentially uniform in these mosaic females. At the level of individual cells, GAD expression was independent of Fmrp. This was shown using monoclonal antibodies to the two isoforms, GAD-65 and GAD-67. Thus, the increased GAD observed in the fragile X mouse appears to be a compensatory response to other changes that are induced by the absence of Fmrp. The changes in the GABAergic system may be a factor in the seizure susceptibility of the fragile X mouse. Decreased inhibitory GABA system activity may also be relevant to the increased prevalence of childhood seizures and hyperactivity in the fragile X syndrome.

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Cellular model of lysinuric protein intolerance. *M.P. Sperandeo[1,2], P. Annunziata[1], P. Piccolo[1], M. Brancaccio[1], G. Parenti[1], S. Fecarotta[1], E. Christensen[3], A. Pota[4], A. Pepe[1], G. Andria[1], G. Sebastio[1].* 1) Dept Pediatrics, Federico II University, Naples, Italy; 2) Dulbecco Telethon Institute, Telethon Foundation, Rome, Italy; 3) Metabolic Laboratory, Department of Clinical Genetics, The Juliane Marie Centre Rigshospitalet, Copenhagen, Denmark; 4) Nephrology Unit, Santobono Hospital, Naples, Italy.

Lysinuric protein intolerance (LPI; MIM 222700) is a congenital amino aciduria caused by a defective transport of cationic amino acids (CAA) at the basolateral membrane of the epithelial cells of intestine and kidney. Clinical manifestations include: vomiting, diarrhea, failure to thrive, visceromegaly, osteoporosis, episodes of coma, mental delay, and severe pulmonary and renal involvements. The molecular basis of LPI is a defective transport of CAA normally exerted by system y+L activity, which is induced by a heterodimer composed of: 4F2hc (gene SLC3A2) and y+LAT1 (gene SLC7A7). We isolated the SLC7A7 gene and characterized 21 causative mutations in LPI patients and identified two independent patients with severe tubular nephropathy. Urines from both patients were collected and cells were selectively grown in an appropriate medium to isolate those of renal tubular origin. CAA transport was tested in these cells and found to be defective as expected in LPI. This is the first LPI cellular model where the functional defect is demonstrated. To investigate the fate of the arginine entrapped intracellularly, the expressions of arginase and iNOS, two key-enzymes of the arginine intracellular metabolism, were studied by Real-Time PCR. The results showed increased expression of both enzymes indicating a constitutional activation of both the early and the late repair phase of the inflammatory response as seen in experimental glomerulonephritis. Interestingly, also the expression of SLC7A6 mRNA, another gene exerting a y+L transport activity at the basolateral membrane, was higher than controls. This might suggest an attempt to restore normal intracellular arginine levels. Acknowledgments: EUGINDAT EC FPVI (EC ref: LSHM-CT-2003-502852) to G. Sebastio and Telethon Foundation (Grant TCP99029) and Compagnia San Paolo to MP Sperandeo.

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Establishment of mouse model for muscle AMP deaminase deficiency. *T. Morisaki[1,2], A. Dohi[1,2], E. Kimura[1], J. Cheng[1], M. Shirai[1], M. Okabe[3], H. Morisaki[1].* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Mol Pathophysiol, Osaka U Grad Sch Pharm Sci, Suita, Osaka, Japan; 3) Dept Exp Genome Res, Genome Info Res Ctr, Osaka U, Suita, Osaka, Japan.

Muscle AMP deaminase (AMPD1) gene is expressed at high levels in skeletal muscle and this enzyme, AMPD, is thought to play an important role in energy metabolism. AMPD1 deficiency, which is one of the most common enzyme defects especially in Caucasian, has been reported to be associated with symptoms of a metabolic myopathy, though not all of individuals with this defect exhibit symptoms. To obtain information of AMPD1 functions in skeletal muscle, we generated knock out mice lacking the muscle isoform of AMPD. Homozygous AMPD1 KO mice showed normal growth and no obvious abnormality. The activity of AMP deaminase in homozygous AMPD1 KO mice was extremely low in skeletal muscle, and mRNA expression or protein expression of AMPD1 was not detected in skeletal muscle of these mice, while other tissues of these mice exhibited normal level of AMPD activity. In skeletal muscle of homozygous AMPD1 KO mice, no histological abnormality or other enzyme defect was found as in that of human individuals with AMPD1 deficiency. In addition, adenine nucleotide level and phosphorylated AMP-activated protein kinase (AMPK) was found to be elevated in skeletal muscle of homozygous AMPD1 KO mice. Based on these findings, AMPD1 KO mice are thought to be a good model for AMPD1 deficiency, and it is indicated that AMPD1 may play an important role for glucose and lipid metabolisms through AMPK pathway as well as nucleotide metabolism.

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Rapid detection of prothrombotic genetic variations of Factor V (G1691A), Prothrombin (G20210A) and Methylenetetrahydrofolate Reductase (C677T) by multiplex single base primer extension and DHPLC. *G. Wu[1], J. Zhu[1], B.L. Wu[2, 3], B. Legendre Jr.[1], S.L. Lilleberg[1].* 1) Translational and Clinical Res, Transgenomic, Inc., Omaha, NE; 2) Department of Laboratory Medicine, Childrens Hospital Boston, Boston, Massachusetts; 3) Department of Pathology, Harvard Medical School, Boston, Massachusetts.

Three allelic sequence variations have been considered as risk factors of hereditary thrombolic disorders: factor V G1691A, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T (MTHFR). We have developed a novel multiplex single base primer extension (PE) protocol in combination with denaturing high-performance liquid chromatography (DHPLC) for simultaneously detecting and genotyping these three genetic variations. A multiplex PCR consisting of three amplicons was designed to amplify the sequences of interest from three target genes. After purification of the PCR product, a multiplex PE reaction, which does not require labeled oligonucleotides, was performed. PE products were then analyzed on the WAVE Nucleic Acid Analysis System under fully-denaturing conditions. The alleles were distinguished by differences in retention time depending on the ddNTP incorporated. Compared to traditional approaches, we demonstrated that this method is rapid, highly accurate and cost effective. It has been successfully validated on patient samples which will be presented.

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Frataxin has a direct role in the mitochondrial electron transport chain. *F. Palau[1], P. Gonzalez-Cabo[1], R.P. Vazquez-Manrique[1,2], S. Ros[1], M.A. Garcia-Gimeno[1], H. Aziz[2], H.A. Baylis[2], P. Sanz[1].* 1) Genetics & Molec Medicine, Inst de Biomedicina, CSIC, Valencia, Spain; 2) Dept of Zoology, Univ of Cambridge, Cambridge, UK.

Frataxin deficiency causes Friedreich ataxia, a neurodegenerative disorder affecting sensitive neurons of dorsal root ganglia and spinocerebellar tracts. Physiological function of frataxin in mitochondria has not been established yet, although several hypotheses have been postulated including mitochondrial iron homeostasis, iron storing, response to oxidative stress, iron-sulphur cluster biogenesis, modulation of mitochondrial aconitase activity and a role in oxidative phosphorylation. We show that frataxin and its *Saccharomyces cerevisiae* orthologue Yfh1p, interact with proteins from the mitochondrial electron transfer chain. We demonstrate that Yfh1p co-immunoprecipitates with yeast succinate dehydrogenase complex subunits Sdh1p and Sdh2p, and with yeast putative orthologues of the electron transfer flavoprotein complex subunits ETFalpha and ETFbeta. Synthetic interaction experiments confirmed a functional relationship between *YFH1* and succinate dehydrogenase genes. We also demonstrate synthetic genetic interaction in *Caenorhabditis elegans* between *frh-1*, the frataxin orthologue gene, and *mev-1*, the nematode gene encoding the succinate dehydrogenase cytochrome b subunit of complex II in mitochondria. We suggest that both yeast frataxin, Yfh1p, and worm frataxin, FRH-1, might regulate the delivery of electrons via complex II towards ubiquinone. We also demonstrate a physical interaction between human frataxin and human succinate dehydrogenase complex subunits, suggesting also a key role of frataxin in the mitochondrial electron transport chain in humans. Consequently, we postulate a direct participation of the respiratory chain in the pathogenesis of the Friedreich ataxia, which we propose to be considered as an OXPHOS disease.

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Peripheral neuropathy with optic atrophy caused by mutations in mitofusin 2. *S. Zuchner[1], P. De Jonghe[2,3], A. Jordanova[2], K. Claeys[2,3], S. Cherninkova[4], V. Guerguelcheva[4], S.R. Hamilton[5], J. Staijch[1], I. Tournev[4], K. Verhoeven[2], M. Pericak-Vance[1], T. Bird[6], V. Timmerman[2], M. Shy[7], J.M. Vance[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Molecular Genetics Department, Flanders Interuniversity Institute for Biotechnology, University of Antwerp, Antwerpen, Belgium; 3) Division of Neurology, University Hospital Antwerpen, Antwerpen, Belgium; 4) Department of Neurology, Medical University, Sofia, Bulgaria; 5) Neuro-ophthalmology Unit, Neuroscience Institute, Swedish Medical Center, Seattle, Washington, USA; 6) Department of Neurology, University of Washington, Seattle, WA, USA; 7) Department of Neurology, Wayne State University School of Medicine, Detroit, MI, USA.

Charcot-Marie-Tooth (CMT) neuropathy with visual impairment due to optic atrophy has been designated hereditary sensorimotor neuropathy type VI (HMSN VI). Sporadic reports of affected families indicated autosomal dominant and recessive forms but the genetic basis of this disease remained elusive. We present five HMSN VI families with subacute onset of optic atrophy and subsequent slow recovery of vision in 70% of the cases. We identified unique mutations in mitofusin 2 (MFN2) in all pedigrees including one nonsense mutation and three de-novo mutations. MFN2 is a mitochondrial fusion factor that was recently reported underlying CMT type 2A. Intriguingly, MFN2 spans the outer mitochondrial membrane and fulfills similar functions in mitochondrial fusion than OPA1, the gene underlying the most common form of autosomal dominant optic atrophy (DOA). We conclude that CMT2A is allelic with HMSN VI both caused by unique mutations in MFN2.

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Targeted deletion of the *Trpm6* gene in mouse results in embryonic lethality. *R.Y. Walder[1], B. Yang[2], M.P. Andrews[1, 4], K.E. Bugge[1], X. Cao[2], J.B. Stokes[3], V.C. Sheffield[1, 4].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Obstetrics and Gynecology; 3) Dept Internal Medicine; 4) Howard Hughes Medical Institute, Iowa City, IA.

In humans, mutations in *TRPM6* cause familial hypomagnesemia with secondary hypocalcemia (HSH) (OMIM 602014). HSH is an autosomal recessive disease that is detected shortly after birth. Affected individuals have severe hypomagnesemia and hypocalcemia, which lead to tetany and seizures, and can be lethal if left untreated. HSH patients with mutations in *TRPM6* have defects in the renal and intestinal transport of magnesium. We have developed a mouse model with a targeted deletion of the *Trpm6* gene. No live mice homozygous for the deletion of *Trpm6*, *Trpm6 (-/-)*, have been detected. Seventeen litters of animals genotyped from intercrosses among mice heterozygous for the *Trpm6* deletion gave an observed F2 ratio for *Trpm6 (+/+)/Trpm6 (+/-)/Trpm6 (-/-)* of 0.38/0.62/0.0, significantly different from the predicted Mendelian ratios of 1:2:1 (^{2} = 32.7, P = 2.4 x 10^{-16}). *Trpm6 (-/-)* mice were present during gestation, up to 18.5 dpc. Expression of *Trpm6* in mouse embryonic RNA samples showed a peak at embryonic day 10.5. Some of the male *Trpm6 (+/-)* animals died within 50 days of birth, suggesting that the *Trpm6 (+/-)* animals might exhibit a difference in Mg handling. Preliminary dietary Mg studies showed that male *Trpm6 (+/-)* animals showed a more rapid decrease in the plasma Mg concentration on a low Mg diet, in comparison to wild type *Trpm6* animals. The plasma Mg levels of the *Trpm6 (+/-)* animals were consistently lower than the wild type *Trpm6 (+/+)* mice ($P < 0.05$). These mutant mice and cell lines derived from them will be useful models for studying the role of *Trpm6* in development, and in the cellular processes of Mg homeostasis.

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Exploring mouse models of human disease in the Mouse Genome Informatics (MGI) system. *H. Dene, C.L. Smith, A. Anagnostopoulos, S. Bello, M. Tomczuk, K. Forthofer, H. Onda, L.L. Washburn, D.L. Burkart, M. Cassell, I. Lu, J.T. Eppig.* Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.

The laboratory mouse is an important model for studying human biology because all life stages are accessible to investigation and there are exceptional genetic tools available for use. These tools include high-resolution genetic maps, myriad inbred strains, a sequenced genome, and technology to specifically alter the mouse genome. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org/) provides integrated access to extensively curated data on the genetics, comparative genomics and mapping, gene expression, mutational phenotypes, strains and tumor biology of the laboratory mouse.

Phenotypic information on mouse mutations, QTL, and strain characteristics is annotated using structured vocabularies that facilitate query-based information retrieval and computational access. Many human conditions are well modeled by engineering mice to carry specific genetic mutations, complex genomic changes, and conditionally expressed genes that have phenotypes comparable to human hereditary diseases. Within MGI, the relationships of mouse genotypes displaying particular phenotypes are curated to the relevant human disease using terms found in Online Mendelian Inheritance in Man (OMIM). These human disease relationships can be searched in MGI from either the perspective of mouse phenotype or human disease. Results of such searches are reported on newly designed web pages that detail the mouse genotype analyzed and human disease for which it is a model. Links are provided to OMIM and to phenotypic allele detail pages where specifics of the phenotypes are described. MGD is supported by NIH/NHGRI grant HG00330.

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Lysinuric protein intolerance: intrauterine growth retardation in Slc7a7 null mice and Igf1/Igf2 expression. *G. Sebastio[1], P. Annunziata[1], G. Corso[2], P. Piccolo[1], M. Brancaccio[1], A. Pepe[1], S. Banfi[3], A. Ballabio[3], G. Andria[1], M.P. Sperandeo[1,4].* 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Department of Biomedical Sciences, University of Foggia, Italy; 3) Telethon Institute of Genetics and Medicine, TIGEM, Naples, Italy; 4) Dulbecco Telethon Institute, Telethon Foundation, Rome, Italy.

Lysinuric protein intolerance (LPI, MIM 222700) is an autosomal recessive defect of cationic amino acid (CAA) transport at the basolateral membrane of epithelial cells of intestine and kidney, caused by mutations of the SLC7A7 gene. The unknown pathogenesis of this multisystem disorder and lack of an effective treatment have prompted the creation of an animal model. A constitutive KO of Slc7a7 was generated by random insertional mutagenesis in ES cells (Lexicon Genetics Inc, Texas). More than 400 Slc7a7+/- intercrosses led to only two Slc7a7-/- live animals. Since birth, both animals showed growth failure compared to siblings. Fed on a low protein diet and citrulline supplementation, the first one survived till 25 months of age, when the treatment was interrupted and replaced by a normal diet. After 2 weeks this animal presented hypotonia, tremors, progressive weight loss and death. The second animal cannibalised their pups and, probably, this overload of proteins caused its death. Metabolic studies confirmed the LPI phenotype: massive urinary CAA and orotic acid excretions. At E 16.5 stage, the proportions of Slc7a7 genotypes were found as expected for an autosomal recessive transmission and the Slc7a7-/- embryos were already smaller than controls. Most of Slc7a7-/- pups were lost at birth because of cannibalism. None of the null embryos showed gross morphological abnormalities apart from a marked delay of renal development. Genes known to play key-roles in the intrauterine growth were tested in SLC7A7-/- embryos and in placentas by Real-Time PCR. The results indicated down-regulation of Igf1, Igf2, Igf2r in SLC7A7-/- embryos and placentas at E. 18.5 stage when compared to controls.
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Introduction of subtle mutations into murine *Smn* exon 7 to alter *Smn* splicing-creating additional animal models for spinal muscular atrophy (SMA). *S. Hammond[1], V. Rao[1], R.G. Gogliotti[1], C. Heier[1], R. Kothary[2], C.J. DiDonato[1].*
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We have sought to create an allelic series of *Smn* mice that would mimic human *SMN2* by producing full-length *Smn (FL-Smn)* transcripts as well as those lacking exon 7 *(7Smn)*. To determine if we could develop this type of animal, we previously generated *Smn* minigenes containing either wild type *Smn* exon 7 or an altered exon 7 containing the CT nucleotide transition found in the *SMN2* gene. When expressed in cultured cells or transgenic mice, the wild type minigene produced only full-length transcripts while the modified minigene alternatively spliced at exon 7 (DiDonato et al., 2001). We also analyzed the regulation of *Smn* exon 7 and demonstrated that it contains an exonic splice enhancer sequence (mESE) that is structurally and functionally similar to the human AG-rich splice enhancer within *SMN* exon 7. Subtle mutations within the mESE, once again in the context of a minigene, caused a variation in the transgene transcript levels which ranged from 0.7% to 83.4%; *FL-Smn* depending on the alteration. Based upon these results, we engineered into the mouse germline two different subtle mutations within *Smn* exon 7. We have now confirmed germline transmission of both alleles and found that these mutations induce *Smn* exon 7 to be alternatively spliced. We will present our results and our characterization of the animals to date. In the long-term these animals will be useful for assessing various treatment modalities for SMA.

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Germline origins of F9 mutation show type-specific sex ratios but no parental age effects. *W.A. Scaringe[1], J. Drost[1], A. Karlea[1], X. Li[1], A. Halangoda[1], K.A. Hill[2], C.K. Kasper[3], J. Liu[4], J. Gill[5], S.S. Sommer[1].* 1) Dept of Molecular Genetics, City of Hope Ntnl Med Ctr, Duarte, CA; 2) Dept of Biology, Univ of Western Ontario, London, Canada; 3) Orthopaedic Hospital, Los Angeles, CA; 4) Beijing Red Cross, Beijing, China; 5) Children's Hospital, Milwaukee, WI.

Germline mutations underlie Mendelian disease and genetic predisposition to complex disease. It has long been hypothesized that most germline mutations occur in the male, and that they increase dramatically with age. These hypotheses can be tested by ascertainment of germline mutation origins, enabling direct estimation of sex ratios of mutation and parental age effects. We ascertained origins of germline mutation in the factor IX gene (F9) by analysis of hemophilia B families. Ascertainment of nearly all hemophilia B probands is enabled by modern medical systems but ascertainment of the other family members needed for molecular determination of the origin is a difficult process which must be carefully monitored for bias. We present analyses of 129 molecularly identified F9 germline mutation origins (65 female, 64 male). The sex ratios of six types of mutations (transitions at CpG, G:C>A:T and A:T>G:C transitions not at CpG, transversions, small and large insertions/deletions) were estimated by two direct methods: by the ratio of maternal grandfather to maternal grandmother origins, and from all origins by Bayesian estimation based on the prior knowledge of the pedigree of each origin family. The results of both methods are similar with the same conclusion: dramatic differences in the sex ratio for different types of mutations. Most single base substitutions occur at significantly higher rates in the male germline. The notable exception is G:C>A:T transitions not at CpG which, like deletions and insertions, occur at about the same rate in males and females. Parental age effects were analyzed by comparing the age of each origin on the date of birth of the child to whom the mutation was transmitted to the population distribution of parental ages in that year. There is no significant skewing to higher age percentiles for either male or female germline origins for any mutation type or all types combined.

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Genetic Modifiers of Severe Liver Disease in Cystic Fibrosis. *K.J. Friedman[1], S.C. Ling[3], E.M. Lange[2], M. Macek Jr[4], A.J. Handler[2], R.G. Pace[2], F. Zou[2], S.C. Bell[5], G. Castaldo[6], F. Salvatore[6], C. Colombo[7], M.J. Phillips[3], J. Zielenski[3], L.C. Tsui[3], M.L. Drumm[8], L.M. Silverman[9], F.A. Wright[2], P.R. Durie[3], M.R. Knowles[2].* 1) Molecular Genetics, LabCorp, RTP, NC; 2) Dep'ts Medicine, Genetics & Biostatistics, UNC-Chapel Hill, NC; 3) Pediatrics, Molecular & Medical Genetics, Univ Toronto, Prog. in Genetics & Genomic Biology, & Prog. in Integrative Biology, Hosp for Sick Children, Toronto; 4) CF Center, Charles Univ, Prague, Czech Republic; 5) Prince Charles Hosp, Brisbane, Australia; 6) CEINGE & Biochimica, Univ Naples, Italy; 7) Pediatrics, Univ Milan, Italy; 8) Pediatrics, CWRU, Cleveland OH; 9) Dep't Pathology, Univ Virginia, Charlotteville VA.

Cystic fibrosis (CF) is complicated by dramatic variation in disease severity & organ involvement among patients. Although some of the variability in pulmonary & pancreatic symptoms is associated with CFTR genotype, it is not known why 2-7% of CF patients develop severe CF liver disease (CFLD), marked by portal hypertension & multilobular cirrhosis. Mutations or polymorphisms in other genes, i.e., those associated with inflammation, hepatic collagen production, immune or cytokine function, may further compound risk for CFLD. We have evaluated 5 genes (1-antitrypsin, angiotensin converting enzyme, glutathione-S-transferase P1, mannose binding lectin 2, transforming growth factor beta-1) previously reported to modify risk for developing CFLD. 133 patients with well-characterized severe CFLD were compared to ~900 controls (CF patients without CFLD, 15 years & older). Initial analyses identified two significant associations. CF patients who carry the AAT-Z allele are more likely to develop CFLD (p=0.0002), an effect most pronounced in females (p=0.0001). Variants in the -509 & codon 10 alleles of TGFB1 suggest an association with CFLD in males (p=0.07 and 0.04, respectively). No significant associations were detected between CFLD & ACE, GSTP1, or MBL2 variants when evaluated individually. More complex analyses are underway including logistic regression analysis & assessment of gene-gene interactions. Evaluation of interplay between gender & CFLD may provide mechanistic insights relevant to diagnostic and therapeutic advancements.

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Expression of hypoxia inducible factor (HIF-1 alpha) and some of its target genes in guinea pig after exchange transfusion with a hemoglobin-based blood substitute. A preliminary investigation. *L. Yeh, P. Buehler, N. Tayebi, A. Alayash.* DH/CBER, FDA, Rockville, MD.

Expression of many mammalian genes is regulated by oxygen (O_2) tension. This study was designed to test the concept that administration of a cell free hemoglobin (Hb) based oxygen carrier (Oxyglobin) in its various oxidative and oxygenation states can be correlated to the expression of HIF-1 alpha, a global transcriptional factor and potentially to other hypoxia sensitive genes such as erythropoietin (EPO), and heme oxygenase (HO-1). We performed 50% and 75% blood / Oxyglobin exchange transfusions in conscious guinea pigs (N = 20). Blood sampling was performed at set times from 0 hr (end of exchange) until 72 hours post end of exchange in. In separate animals, following euthananasia at set times (0-72 hrs), the right and left kidneys were harvested, immediately frozen in liquid nitrogen followed by total RNA extraction using Trizol. The cDNA for HIF-1a, EPO and HO-1 were generated by reverse transcription with primer pairs designed and synthesized based on human HIF-1 alpha gene sequence. By semiquantitative RT-PCR, mRNA of HIF-1 alpha were detected using a nested PCR technique. Genes were amplified using PCR and the products were separated using 1.5% agarose gel. The oxygenation and redox states of the Hb in plasma obtained was monitored by spectrophotometry and the plasma disappearance of Oxyglobin monitored via HPLC. O_2 carrying ferrous Hb (HbFe2+) monitored in plasma following transfusion declined linearly as a function of time. This was coupled with a proportional elevation of the ferric (HbFe3+) or non-O_2 carrying form of Hb, indicating a loss of the oxygen carrying capacity. The expression of HIF-1 alpha was increased three-fold over baseline (Sham = surgery + recovery + sacrifice) at 4 hours post exchange transfusion and this level of expression was sustained until 72 hr. Exchange transfusion with a non-oxygen carrying oncotically matched Albumin and Oxyglobins ferric form result in higher expressions of HIF. The levels of both EPO as well as HO-1 mRNAs were also elevated in the kidney tissues of these animals.

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Differential gene expression profiles unraveled by microarray analysis in periosteal fibroblasts deriving from Apert syndrome patients. *R.D. Fanganiello[1], A.L. Sertie[1], E.M. Reis[2], N. Oliveira[1], F. Kok[3], S. Cavalheiro[4], N. Alonso[3], S. Verjovsk[2], M.R. Passos[1].* 1) CEGH-IBUSP, Brazil; 2) IQ-USP, Brazil; 3) FMUSP, Brazil; 4) UNIFESP, Brazil.

Several signaling pathways can be initiated by ligand activation of fibroblast growth factor receptors (FGFRs), leading to phenomena such as cell proliferation, differentiation and apoptosis, which are critical in skeletal development. FGFR2 gene mutations with dominantly acting mechanisms are responsible for a variety of craniosynostosis syndromes. The most severe of the craniosynostosis is known as Apert syndrome (AS), essentially characterized by premature closure of coronal sutures, severe syndactyly of hands and feet and mental retardation. Almost in all cases of AS, FGFR2 ligand biding affinity and specificity are respectively enhanced and violated by one of two gain of function missense mutations, S252W or P253R. It is known that AS osteoblast mutations cause overexpression of PKC pathway, increased ossification and apoptosis, but the abnormal FGFR2 signaling is not fully understood. To gain further information concerning the signaling pathways and transcription factor networks unchained by FGFR2 activation we are examining, through oligonucleotide microarrays technology, the gene expression profile of primary cultured fibroblasts from the coronal suture periosteum of four AS patients (S252W mutation) and comparing them to those of four wild type cultured periosteal fibroblasts. We detected expression of *FGFR2* gene in these cells by RT-PCR and the protein was detected by immunocytochemical and western blot experiments. In this study we used a batch of 8 oligonucleotide arrays (CodeLink system) representing 20,000 nonredundant human genes. After data mean normalization, with p values 0.05, changes in the expression profile were found in 131 cDNAs, and using the Expression Analysis Systematic Explorer (EASE), the category cell cycle seems to be an enriched biological theme. Among them 20 genes showed signal/noise ratios (SNR) |1.5|. To further investigation we intend to use quantitative RT-PCR and RNA interference experiments. CEPID / FAPESP; CNPq robertofanganiello@yahoo.com.

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Mutation in NIZP1, a NSD1-interacting zinc finger protein, as a novel cause of Sotos-like conditions. *G. Borck[1], A.L. Nielsen[2], P. Edery[3], D. Sanlaville[1], A. Munnich[1], R. Losson[4], V. Cormier-Daire[1], L. Colleaux[1].* 1) INSERM U393, Hosp Necker-Enfants Malades, Paris, France; 2) Department of Human Genetics, Aarhus University, Aarhus, Denmark; 3) Clinical Genetic Service, Lyon, France; 4) IGBMC, Illkirch, France.

Sotos syndrome is an overgrowth condition characterized by pre-and postnatal overgrowth, macrocephaly, variable degree of mental retardation and typical facial features. Haploinsufficiency caused by whole gene deletions and point mutations of the NSD1 (Nuclear receptor SET domain containing protein 1) gene, a SET-domain histone lysine methyltransferase involved in transcriptional regulation account for 70% of cases. However the disease causing mechnanism in other cases remain unexplained.

A novel NSD1-interacting zinc finger protein: NIZP1 has been recently identify using NSD1 as a bait in a yeast two-hybrid screen. This protein belongs to the SCAN-KRAB subfamily of C2H2-type zinc finger proteins.

Owing to the functional relationship between the two proteins, we investigated whether unexplained cases of Sotos or Sotos-like syndrome could be related to NIZP1 anomalies. 44 patients were first tested for deletion of the NIZP1 gene using polymorphic markers genotyping but, in all cases, a microdeletion encompassing NIZP1 was excluded. The eight coding exons of the NIZP1 gene were subsequently sequenced. We identified a missense mutation (R72Q) which occurs de novo and was not found in 226 unrelated control individuals. Interestingly, this mutation alters an highly conserved amino-acid located within the SCAN domain, a region of the protein which mediates dimer formation between SCAN-domain containing proteins.

These data suggest that NIZP1 mutations may account for some cases of unexplained overgrowth syndromes and provide new evidence for the role of the NSD1/NIZP1 pathway in growth regulation .

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Interaction of fibroblast growth factor and C-natriuretic peptide signaling: implications for achondroplasia. *W.R. Wilcox[1], P. Krejci[1], B. Masri[2], V. Fontaine[2], P.B. Mekikian[1], M. Weiss[3], H. Pratts[2].* 1) Med Genet Inst, Cedars-Sinai Med Ctr, Los Angeles, CA; 2) INSERM U589, Institut Louis Bugnard, Toulouse, France; 3) Univ Wash, Seattle, WA.

Activating mutations in fibroblast growth factor receptor-3 (FGFR3) result in several forms of human dwarfism including achondroplasia. Although the mechanism of FGFR3 action remains obscure, both chondrocyte proliferation and differentiation appear to be disturbed by aberrant FGFR3 signaling. Several reports have shown that sustained activation of the Erk MAP kinase pathway accounts for the inhibitory effect of FGF signaling on chondrocyte proliferation. Furthermore, the murine achondroplasia phenotype can be partially rescued by over-expression of C-natriuretic peptide (CNP) in cartilage.

We studied the interaction of fibroblast growth factor (FGF) and CNP signaling in chondrocytes. CNP antagonized FGF2-induced growth arrest of rat chondrosarcoma (RCS) chondrocytes by inhibition of the Erk MAP kinase pathway. This effect of CNP was protein kinase G-dependent and was mimicked by the cGMP analog pCPT-cGMP. We found that CNP blocks the Erk pathway at the level of Raf-1. CNP also counteracted the FGF2-mediated degradation of the RCS extracellular matrix. CNP partially antagonized FGF2-induced expression, release and activation of several matrix metalloproteinases. In addition, CNP compensated for the FGF2-mediated matrix loss by upregulation of matrix production independent of its interference with FGF signaling.

FGF signaling thus affects chondrocyte behavior by inhibition of proliferation and degradation of the extracellular matrix. CNP can reverse both phenotypes by inhibiting the Erk pathway and independently increasing extracellular matrix synthesis. Thus, CNP utilizes both direct and indirect ways to antagonize the outcomes of FGF signaling in chondrocytes. Pharmacologically increasing cGMP in cartilage may represent a therapeutic approach for achondroplasia.

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Efficiency of splicing inversely correlates with clinical severity in dystrophinopathy due to deep intronic splice site activation mutations. *K.M. Flanigan[1,2,3], C.B. Anderson[1], K.J. Hart[1,2], M.T. Howard[1], T. Tuohy[1].* 1) Dept of Human Genetics, Univ Utah, Salt Lake City, UT; 2) Dept of Neurology, Univ Utah, Salt Lake City, UT; 3) Dept of Pathology, Univ Utah, Salt Lake City, UT.

Becker and Duchenne Muscular Dystrophy (BMD and DMD) can be caused by deep intronic mutations which create splice sites resulting in the inclusion of pseudoexons in the DMD mRNA. Such mutations account for at least half of the 5-7% of mutations not detected in lymphocyte-derived DNA samples by modern mutation detection methods. We detected two such mutations in patients with dystrophinopathy. One patient, with BMD, carries an intron 11 mutation (c.1331+17770C>G) which creates a splice donor site, resulting in the incorporation of a 79 nucleotide (nt) out-of-frame insertion. A second patient, with DMD, carries an intron 45 mutation (c.6614+3313G>T), resulting in a 139 nt out-of-frame insertion. We hypothesized that the milder phenotype may result from less efficient alternate splicing. In order to investigate the relationship of splicing efficiency to phenotype, quantitative RT-PCR was performed using forward amplification primers which span the junction between the wild type exon (11 or 45) and the pseudoexon (11a or 45a) and compared to amplification with primers spanning the wild-type (11-12 or 45-46) junction. Each reaction product was normalized against the EEF gene, and compared to results found in normal control muscle tissue. In the DMD sample, the alternatively-spliced transcript was present at the level of 41% compared to a normal control, and no wild-type transcript was detected. In the BMD sample, the alternatively-spliced transcript was present at the level of 40%, but the wild type transcript was detected at a level of 13%. These results suggest that in the setting of cryptic splice site mutations, clinical phenotype is dependent upon splicing efficiency, which can be determined using quantitative RT-PCR methods.

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Transgenic mouse model faithfully reproduces clinical symptoms of LGMD1A. *S.M. Garvey[1], D.R. Claflin[2], J.A. Faulkner[2], M.A. Hauser[1].* 1) Duke University, Durham, NC; 2) University of Michigan, Ann Arbor, MI.

The myotilinopathies are a group of autosomal dominant, adult-onset, progressive, and clinically heterogeneous muscle disorders that includes both Limb-Girdle Muscular Dystrophy Type 1A (LGMD1A) and a subset of myofibrillar myopathy (MFM) patients. Seven independent missense mutations in the *myotilin* gene have been characterized in LGMD1A and MFM patients. We generated transgenic mice expressing the LGMD1A thr57ile *myotilin* mutation (TgT57I) specifically in skeletal muscle. The transgene includes the human myotilin cDNA and an N-terminal c-myc epitope tag. Three independent lines of TgT57I mice develop progressive myofibrillar pathology. Sections of multiple muscles show an age-dependent increase in the size and number of dense myofibrillar plaques or aggregates. Ultrastructural analysis reveals rimmed vacuoles, Z-disc streaming, and large patches of myofibrillar disarray similar to those seen in human patients. Using a c-myc antibody, we show that the TgT57I product localizes properly to the Z-disc, yet also localizes heavily to the myofibrillar aggregates. A similar pattern of immunoreactivity is observed for endogenous myotilin, -actinin, -filamin, and desmin, reproducing the aggregate staining seen in MFM patients. Ubiquitin also localizes to the aggregates, suggesting a role for the proteasome in aggregate clearance. Intriguingly, the integral sarcomeric protein, titin, does not localize to the aggregates. To further characterize the TgT57I mice, we performed whole muscle physiology. The EDL muscles mass is reduced by 33%, its cross-sectional area is reduced 30%, and its maximum specific force is reduced 23%. In contrast to the largely fast-twitch EDL muscle, TgT57I soleus muscle physiology remains normal; the soleus is also spared of any myopathology. The slow-twitch fiber composition of soleus may protect it from the toxic accumulation of aggregates. Similarly, in LGMD1A and MFM patients, the muscle group specificity of pathology and weakness may be due to the underlying fiber type composition. We will also discuss single-fiber physiology and the effect of treadmill exercise on the pathology observed in this promising mouse model of LGMD1A.

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Possible common founder effect of the G526R mutation in 4 dSMA-5 Algerian Sepharadic Jewish families. *H. Azzedine, O. Dubourg, D. bouteiller, R. Benyaou, A. Brice, E. Leguern.* INSERM U679 (exU289)-NEB, Hosp de la Salpetriere, Paris, France.

CMT is a pathological and genetic heterogeneous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished on anatomopathological and electrophysiological grounds: demyelinating and neuronal CMT. Up to now, 5 different loci have been reported and 4 genes have been identified in neuronal autosomal dominant forms of the disease. Recently, the GARS (Glycyl tRNA synthetase) gene has been implicated in distal spinal muscular atrophy type V (dSMA-V) and CMT2D both disease mapped to 7p14 chromosome. We screened eight families with neuronal CMT for 7 microsatellites markers covering the the 7p14 locus. Assignment of the families for this locus was established by halotype segregation. Three families Algerian of Sepharadic Jewish nacestry were putatively linked to the CMT2D/dSMA-V locus. Sequencing of the 17 exons of GARS gene was performed in all examined consent individuals of the 3 families. We identified a unique G526R mutation that segregated with the same haplotype in all 3 families. This mutation was previously reported in the literature in another Algerian Sepharadic Jewish family. The common origin of the 4 families with the same mutation and a common hapltotype segregating with this mutation in our 3 families suggest a founder effect in the Algerian Jewish ethnic group.

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Development of a high-throughput DNA microarray-based resequencing system for comprehensive mutational analysis for hereditary and sporadic spastic paraplegias. *H. Ishiura, Y. Takahashi, J. Goto, S. Tsuji.* Department of Neurology, The University of Tokyo, Tokyo, Japan.

[Background] Spastic paraplegias (SPGs) are heterogeneous neurodegenerative disorders characterized by progressive lower limb spasticity and pyramidal weakness. Up to present, SPG1-28 have been known for the loci for hereditary spastic paraplegia(HSP), and causative genes have been identified for *SPG1, 2, 3A, 4, 6, 7, 10, 13 and 20*. However, little is known about genotype-phenotype correlation. In addition, a substantial number of sporadic cases present with clinical manifestations similar to those of hereditary cases. Since it is difficult to make a correct diagnosis only from clinical information, we are confronted with a strong demand for a high-throughput system for mutational analyses. The purpose of this study is to design a system and to elucidate molecular epidemiology of SPGs in the Japanese population. [Method] We have designed a high-throughput DNA microarray system based on GeneChip^{TM} CustomSeq resequencing system (Affymetrix), which is capable of analyzing complete nucleotide sequences of all the exons and splicing junctions of 5 causative genes (*SPG1:L1CAM, SPG2:PLP1, SPG3A:atlastin, SPG4:spastin and SPG7:paraplegin*). The study includes 7 patients with autosomal dominant inheritance (ADHSP), 3 patients born to consanguineous parents, one patients with undetermined mode of inheritance, 2 cases with Pelizaeus-Merzbacher disease (PMD) and 16 sporadic patients. [Results] We identified 3 *SPG4* mutations (Q178X, I440V, Q280X) among the ADHSP patients, and one *PLP1* mutation (W145X) in a patient with PMD. In addition, we found one *SPG4* mutation (K502X) in a sporadic case. [Discussion] Our findings indicate that SPG4 is the most frequent HSP in the Japanese population, which is compatible with previous reports on other ethnic populations. We furthermore found 1 *SPG4* mutation in an apparently sporadic case, raising a possibility that some of the sporadic cases are caused by mutations of *SPG4* with a reduced penetrance. The microarray resequencing system is comprehensive and highly efficient for mutational analyses of disorders with such tremendous locus heterogeneity.

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Allele specific analysis of familial pulmonary arterial hypertension patients reveals pathogenic defects not detected by DNA mutation screening. *V. James, M.A. Aldred, R.D. Machado, R.E. Harrison, R.C. Trembath.* Dept. Genetics, Univ Leicester, Leicester, United Kingdom.

Heterozygous mutations in the gene bone morphogenetic protein receptor II (*BMPR2*) underlie familial pulmonary arterial hypertension (FPAH). Some 70% of *BMPR2* mutations introduce a premature termination codon (PTCs) that results in transcripts likely to be rapidly degraded by the nonsense mediated decay pathway (NMD). Direct sequencing of coding regions and exon-intron junctions of genomic DNA results in approximately 30% of FPAH index cases without identified mutations despite evidence of linkage to the *BMPR2* locus.

We developed an allele specific assay to quantify RNA transcript levels to search for defects in *BMPR2* expression. Lymphocyte mRNA was extracted from five FPAH subjects, 2 with known mutations (IVS7968+3delA and deletion of exon 2) and 3 without detected mutations. Allelic imbalance (quantitative reduction in allelic expression) was detected for informative subjects with known mutations but also in an FPAH cases without. To further elucidate the mechanism leading to allelic imbalance we analysed a patient derived lymphoblastoid cell line. Allele specific expression was quantified before and following treatment with the protein synthesis inhibitor, puromycin. Preliminary data shows a significant increased in the abundance of transcripts derived from the risk allele suggesting that NMD explains the steady-state difference leading to allelic imbalance. Direct sequencing of a 2.2kb region 5 of the translation start site identified a substitution -944/5GC-AT, which was not seen in 160 control chromosomes.

These data suggest NMD leads to degradation, not only of nonsense *BMPR2* transcripts but also non-coding mutations and supports haploinsufficiency as the predominant molecular mechanism predisposing to FPAH.

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Mutations in the gene for ichthyin, a minor gene for congenital ichthyoses, found in patient samples and also in controls. *K.M. Eckl[1], W. Küster[2], H. Traupe[3], H.C. Hennies[1].* 1) Cologne Center for Genomics, University of Cologne, Germany; 2) TOMESA Clinic, Bad Salzschlirf, Germany; 3) Dept Dermatology, University of Munster, Germany.

Autosomal recessive congenital ichthyoses (ARCI) form a group of rare, severe disorders of keratinization with a prevalence of 1 in 100.000 to 200.000 in the European population and in the US. Both phenotypes and genetic etiology are extremely heterogeneous. Up to date six different gene loci were described: *TGM1* on 14q11.2, *ALOX12B* and *ALOXE3* on 17p13.1, *ABCA12* on 2q34-q35, and the gene for ichthyin on 5q33. Two loci were described on chromosome 19 but corresponding mutations underlying ARCI are still unknown. Here we describe the analysis of a collection of 118 non-related ARCI patients for mutations in the gene for ichthyin described recently. All samples were previously checked to be free of any mutation in one of the genes *TGM1*, *ALOX12B*, or *ALOXE3*. In 15 families we have found four different mutations, whereof one is a nonsense mutation and three are missense mutations. Two mutations are novel, two further mutations, Arg83X and Ala114Asp, were described before in patients from consanguineous families of Turkey, Algeria, and Colombia, whereas our patients originate mainly from Germany and Turkey. All patients but two showed homozygosity for the respective mutations, although no consanguinity was reported. Healthy controls showed neither the mutation Arg83X nor two of the missense mutations. In contrast, the mutation Ala114Asp, which we have found in ichthyosis patients from 11 families and which was described in patients from 7 families before, was also present in 4 of 100 ethnically matched control chromosomes (4%), indicating that this mutation is most probably a polymorphism. In summary, we have found potentially pathogenic mutations in 3% of our ichthyosis patients. Thus we conclude that the gene for ichthyin is an uncharacterized, minor ARCI gene. Functional analysis of this gene must be the next task in order to further analyze the impact on ichthyosis. As a first step, we have cloned the gene and determined the genomic organization, which has still been equivocal until now.

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A novel GH-1 gene splicing mutation in an Italian family with Isolated Type II Growth Hormone Deficiency (IGHD II). *P. Momigliano-Richiardi[1], D. Vivenza[1], L. Guazzarotti[2], B. Di Natale[2], M. Godi[1], S. Mellone[1], G. Bona[1], M. Giordano[1].* 1) Dept Medical Sci, Eastern Piedmont Univ, Novara, Italy; 2) Dept of Pediatrics, L. Sacco Hospital, University of Milan, Milan, Italy.

A new mutation responsible of IGHD-II was detected in a 9 month old girl (the proband) with severe growth failure (-5.8 SDS) and GH peaks after stimulus < 0.4 ng/ml, and her mother (-6.9 SDS and GH peaks < 0.5 ng/ml). Sequencing of the entire GH1 gene in the proband evidenced an heterozygous 22 bp deletion in IVS3 (+54-75) encompassing the branch site. RT-PCR of her peripheral blood mRNA showed both a normal length fragment, corresponding to the wild-type allele, and an equally intense shorter band corresponding to the IVS3 +54-75 allele that, when sequenced, revealed exon 3 skipping. Its predicted product is a shorter protein lacking aminoacids 32-71, corresponding to the 17.5 kDa isoform physiologically present at a very low dose. The same IVS3 mutation was present in the proband's mother and absent in her normal stature relatives. Haplotype analysis in all the family members using GH1 promoter SNPs revealed that this was a de novo mutation in the proband's mother. To better evaluate the effect of the IVS3 +54-75 on mRNA splicing, an expression construct containing the GH1 allele with the IVS3 deletion was transfected into GHC4 rat pituitary cells. The mutated allele produced at least four differently spliced products: the full-lengh cDNA, visible as a weak band, the exon 3 skipped mRNA and two new aberrantly spliced products correponding to the GH1 mRNA deleted of the first 86 bases of exon 4 (leading to a premature stop codon) and to an mRNA lacking the entire exon 4 (leading to a protein lacking aa 72-126). Thus the 22 bp deletion has two main effects: 1) Exon3 skipping, as observed in all the previously described cases of IGHD-II. This effect is likely caused by the relevant reduction in size of the IVS3 (Ryther et al, 2004). 2) Partial or total exon 4 skipping, likely due to the depletion of the branch site that does not allow the use of the IVS3 acceptor splice site. The consequence is either the activation of a criptic splice site in exon 4 or the total skipping of exon 4.

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A comparison of mutation patterns in suspected cases of congenital long QT syndrome and controls. *M.S. Pungliya[1], B.A. Salisbury[1], R.S. Judson[1], D.J. Tester[2], M.L. Will[2], M.J. Ackerman[2].* 1) Computational Genomics, Genaissance Pharmaceuticals, New Haven, CT; 2) Mayo Clinic College of Medicine, Rochester, MN.

Congenital long QT syndrome (LQTS) affects about one in 5000 individuals. LQTS is a heritable condition often characterized by prolonged QT intervals as seen by a 12-lead electrocardiogram. Although nearly half of susceptible hosts experience a lifelong asymptomatic course, LQTS can present with sudden cardiac death even in infancy. Among the most definitive cases, about 75% are found to have rare or unique protein-altering mutations in the cardiac ion channel genes KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2; hundreds of such mutations have been reported, few of which have been observed in healthy controls. Here, we compared the patterns of mutation discovery in 611 healthy controls with that of 541 cases published in a recent compendium. While 50% of the published cases had protein-altering mutations in one of the five LQTS causing genes, we found that almost 5% of the controls also had such mutations. In addition to the overall rates, the relative numbers of mutations among the five genes was also significantly different between cases and controls ($p = 5.9 \times 10^{-8}$), as seen in the table below. From these relative rates, it is clear that causality can be confidently assigned to case mutations found in KCNQ1 and KCNH2, and less so for SCN5A. Further, when a case has two mutations, we can be confident that at least one of the mutations is causative, but we can estimate that for half of those cases, one of the mutations may represent a rare but clinically insignificant background variant.

Percentages and counts among Cases and Controls in the 5 genes.

	KCNQ1	KCNH2	SCN5A	KCNE1	KCNE2	Multiple Mutations
Controls (611)	1.0% (6)	1.1% (7)	2.3% (14)	0.3% (2)	0.5% (3)	0.2% (1)
Cases (541)	22.2% (120)	17.2% (93)	4.8% (26)	0.6% (3)	0.2% (1)	5.4% (29)

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Clinical Molecular Diagnosis of Hypertrophic Cardiomyopathy: the First 50 Cases. *M. Peng, E. Duffy, H. Rehm, P. Verlander, V. Joshi, R. Madore, J. Seidman, C. Seidman, C. Ho, R. Kucherlapati.* Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Cambridge, MA.

Hypertrophic cardiomyopathy (HCM) is a primary disorder of the myocardium with a genetics base. The prevalence of the disease is estimated to be one in 500. At least 14 genes have been reported to cause the disease. More than 200 sarcomere mutations have been found in 50-70% of the HCM patients. Molecular diagnosis has been a challenge because of genetic and allelic heterogeneity. Laboratory for Molecular Medicine at Harvard Partners Center for Genetics and Genomics has offered a sequencing based mutation detection test for more than a year. Five sarcomere genes (MYH7, MYBPC3, TNNT2, TNNI3 and TPM1) were included in this test. The first 50 individuals tested are reported here. 17 out of 31 (54.8%) probands were positive for at least one mutation among the five major sarcomere genes. 3 out of 17 (17.6%) probands have two mutations. 3 out of 17 (17.6%) families with a mutation positive probands have sent other family members to test. In two families, the novel changes were found to be co-segregated with the disease. Altogether 20 different mutations (13 in MYBPC3, 4 in MYH7. 2 in TNNT2 and 1 in TNNI3) were identified. Fifteen (75%) were novel mutations that have not been described previously in the literature or the mutation database (http://cardiogenomics.med.harvard.edu). In conclusion, a comprehensive analysis of genes implicated in HCM continues to be necessary to identify the private mutations uncovered in clinically affected individuals. More than half of the mutations in clinical samples have never been reported despite the fact that more than 800 HCM probands have been genetically screened in research studies worldwide. A cost-effective DNA diagnostic test to scan every single base of the major HCM genes for the private mutations of the probands followed by a known mutation testing for the family members, is the appropriate genetic test approach with our current knowledge of the sarcomere gene mutations.

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Clinical and genomic data bank of subjects with sporadic and familial cavernous angiomas of the nervous system of Italian origin. *F. Gianfrancesco[1], V. Maglione[2], T. Esposito[1], M. Cannella[2], C.L. Liquori[3], D.A. Marchuk[3], F. Squitieri[2].* 1) Institute of Food Science, National Research Council, Avellino, Italy; 2) Neurogenetics Unit IRCCS Neuromed, Pozzilli (IS), Italy; 3) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cavernous angiomas of the nervous system (CCMs) are cerebral/medullar y malformations causing intracerebral haemorrhage, seizures, headache and focal neurological symptoms. During these last years, the linkage analysis approach allowed the discovery of three different loci on chromosomes 7q21.2 (CCM1), 7p13 (CCM2) and 3q25.2-q27 (CCM3). The DNA screening of the mapped genes highlighted a number of mutations in KRIT1 (CCM1), MGC4607 (CCM2) and PDCD10 (CCM3). We set up a clinical and genomic bank with patients and families affected with CCMs, all of Italian origin, at the Neurogenetics Unit of Neuromed and started to screen the DNA of familial and sporadic affected subjects. In total, we have so far tested subjects from five unrelated families and 6 sporadic cases. Family CAV01, which has previously contributed to the identification of CCM2 locus and whose affected people had CCMs confined to brain, showed a frameshift mutation (56delG) in the exon 2 of MGC4607. Family CAV17 showed a new KRIT1 mutation, never reported yet, changing a CAA triplet in 238 with a TAA stop codon. Such non sense mutation caused a phenotype, variably expressed among the subjects, characterized by cerebral, medullary and cutaneous angiomas. Interestingly, this family generated a number of consanguineous marriages potentially generating homozygosity for mutation and an increased concentration of cases in that particular geographical area. Family CAV02 was analyzed for all three gene mutations and is presently contributing to the identification of a fourth gene locus on chromosome 3q. Two more CAV families from our bank have been screened and mutations in all the three CCM genes excluded, thus potentially of help for further identification of new genetic loci.

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Three severe cases of EBS Dowling-Meara caused by missense and frameshift mutations in keratin 14 gene. *J. Mazereeuw-Hautier[1], M. Titeux[2], S. Hadj-Rabia[3], C. Prost[4], L. Tonasso[2], C. Bodemer[3], A. Hovnanian[2,5].* 1) Service de Dermatologie, Purpan Hospital, Toulouse, France; 2) INSERM U563, Purpan Hospital, Toulouse, France; 3) Service de Dermatologie, Saint Louis Hospital, Paris, France; 4) Service de Dermatologie, Necker Hospital, Paris, France; 5) Service de génétique médicale, Purpan Hospital, Toulouse, France.

Epidermolysis bullosa simplex (EBS) is a group of blistering diseases classified into three major subtypes caused by mutations in the genes encoding keratin 5 (KRT5) and keratin 14 (KRT14). In this study, we analysed three patients affected with an unusually severe form of EBS Dowling-Meara type (EBS-DM), with early and marked hyperkeratosis. Sequencing of the KRT14 gene revealed a heterozygous mutation p.M119T in two of the three patients. Amino acid 119 in keratin 14 is located within the highly conserved Helix Initiation Peptide at the a position of the first heptad and is predicted to face, at the d position, leucine 176 of keratin 5. The conversion of this hydrophobic residue into a threonine, which is hydrophilic, is predicted to destabilise the helix and impair coiled-coil interactions. Interestingly, the same mutation was previously reported in two severe cases of EBS-DM, whereas mutations, conserving the hydrophobicity of this residue (p.M119V and p.M119I) led to milder phenotypes. In the third patient, a novel c.1246delC mutation was identified. This mutation, located at the very beginning of the Helix Termination Peptide, leads to a frameshift and to a premature termination codon, predicting the synthesis of a 25 amino acid missense carboxyterminal sequence. The keratin 14 tail domain, which is replaced by an aberrant sequence, is thought to be important for the bundling of the filaments. Since a previously described K14 tailless mutant was not associated with severe EBS-DM we suggest that the severity of the phenotype may result from the presence of the abnormal K14 tail. Accordingly with the EBS-DM diagnosis, keratin aggregates were found in basal keratinocytes of two of the three patients These cases illustrate the extreme severity of some EBS-DM at the neonate period and further document genotype-phenotype correlations.

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New BBS mutations in known BBS genes. *H. Dollfus[1], C. Stoetzel[1], V. Laurier[1], D. Bonneau[2], A. Verloes[3], A. Megarbane[4], F. Perrin-Schmitt[1], JL. Mandel[5], M. Cossee[1,6].* 1) Laboratoire de génétique médicale EA,Faculté de médecine-ULP, Hôpitaux Universitaires,Strasbourg, France; 2) Service de génétique, CHU Angers, France; 3) Hôpital Robert Debré, Paris, France; 4) Université Saint-Joseph, Beirut, Lebanon; 5) IGBMC, Illkirch, France; 6) Laboratoire de diagnostic génétique,Strasbourg, France.

Introduction

Bardet-Biedl syndrome (BBS) is a genetically heterogenous condition characterized by retinitis pigmentosa, obesity, polydactyly, renal and genital malformations and learning disabilites. To date 8 genes have been identified (BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7 and BBS8) and triallelic inheritance demonstrated for some families. A cohort of BBS families is being explored for all the BBS genes (Hichri et al, 2005). We present herein 12 new BBS mutations.

Families and methods:

We are studying a large cohort with BBS for which the analysis of 40 families has revealed new BBS mutations.

Results:

Although screening for all the families is not completed in extenso we report on twelve previously unreported BBS mutations. BBS2: C307Y; BBS3: R122Q; BBS4: Q247X (homozygous); BBS5: 123 delA - K41fsX52 (homozygous); BBS6: I297T ; BBS7: 340A>G, M114V splicing mutation, 52843;1G>A - splicing mutation , S574C, 1967-1968delTAinsC - L656fsX673 (long isoform) L656fsX678 (short isoform), BBS8: 459G>A - T153T splicing mutation (homozygous) , 594 43;1G>A - splicing mutation (homozygous), 59443;1-2delGT - splicing mutation.

Conclusions:

Summarizing the litterature and our results recurrent mutations in BBS seems to be rare. An excess of heterozygous mutations are reported herein (8/12) and this is in favor of the triallelic possible inheritance for these families. Reporting on new mutations in BBS is important in the perspective of setting micro-array based mutation analysis.

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Screening for DJ-1 mutations in early onset PD. *P. Tarantino, F. Annesi, F.E. Rocca, P. Spadafora, I.C. Cirò Candiano, S. Carrideo, D. Civitelli, E.V. De Marco, G. Annesi.* Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy.

Mutations in DJ-1 gene cause a clinically characteristic autosomal recessive juvenile onset form of Parkinsons disease (PD). Such mutations are thought to lead to a loss of DJ-1 function. DJ-1 is composed of eight exons; the open reading frame is encoded within exons 2-7, while exon 1 is alternatively spliced (1a/b). In this study we analysed the DJ-1 gene in 40 sporadic patients with early onset PD (age of onset 45 years of age) and 100 appropriate controls, originated from Southern Italy. These patients were evaluated previously for the presence of Parkin and PINK1 mutations and found to be negative. DNA was extracted from peripheral blood using standard protocols. Exons were amplified and obtained fragments were directly sequenced on ABI3100 automated DNA sequencer. Absolute quantification was performed by real time PCR 7900 HT-SDS, using TaqMan probes for exons 2,3 and 7. For remaining exons 1,4,5 and 6 we set up a Sybr Green assay. In a patient with an age at onset of 35 years, we detected an heterozygous nucleotide change (CG) in intron 1 (nucleotide 10703, ref. AL034417) and a single heterozygous deletion mutation (IVS4+3insA) in intron 4 splice site. These novel variants were not found in 100 control subjects. In the same patient genomic rearrangements were excluded by absolute quantification in real time PCR. By sequence analysis, no mutations were found in remaining patients. Nevertheless, absolute quantification is still to be performed. The IVS4+3insA mutation results in a single nucleotide insertion in a conserved sequence of the invariant AG splice acceptor site of intron 4. We hypothesized that this variant could have consequences on DJ-1 RNA, leading to an aberrant transcript. Moreover, the detected intronic change is potentially functional because it is localized 159 bp after the transcriptional activate site and approximately 42 bp downstream of an SP1 binding site. Synergic action of both heterozygous variants could account for clinical features of examined patient.

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Mutation analysis in nephronophthisis (NPHP) applying a combined approach of homozygosity screening and heteroduplex cleavage with CEL I nuclease. *E. Otto, J. Helou, S. Allen, F. Hildebrandt.* Dept Ped, Univ Michigan, Ann Arbor, MI.

NPHP is an autosomal recessive kidney disease that causes chronic renal failure in children. Five genes (NPHP1-5) have been identified to date. Tubular basement membrane disintegration, fibrosis, and cyst formation are the prominent features. We report here on a straightforward and inexpensive method of mutation screening in a worldwide cohort of 645 unrelated patients to identify homo- and hemizygous mutations in this disease entity. First, homozygous deletions of NPHP1 were detected in 152 NPHP-families (24%) by a multiplex PCR approach. In order to identify additional families with point mutations in NPHP1 or NPHP4, we screened one affected member of each of 400 NPHP families for homozygosity of 3 consecutive highly polymorphic microsatellite markers within the NPHP1 (common 250 kb deleted region) and NPHP4 loci. As a result, 64 (10) patients showed homozygosity for all markers tested in the NPHP1 (NPHP4) region. Thereafter, we PCR amplified each of the 20 (30) exons of the NPHP1 (NPHP4) gene along with their exon/intron boundaries. These samples were combined with wildtype PCR products and heteroduplex formation was performed. Using single strand specific nuclease CEL I (crude celery plant extract) mismatch cleavage was performed at 45C for 5 min and analyzed on an agarose gel for aberrant bands. The CEL I enzyme recognizes single base mismatches present in the heteroduplex DNA and cleaves both strands. In order to test the sensitivity of the CEL I enzyme we digested the exon PCR products of formerly published mutations and were able to detect 73 out of 79 different mutations (92%). Sequencing of the CEL I detected exons revealed new NPHP1 point-mutations in 14 patients and homozygous NPHP4 mutations in 8 patients. Furthermore, we used the CEL I enzyme mutation approach to analyze 20 patients with infantile NPHP (end stage renal disease before age 5 years) and 10 patients with NPHP combined with Retinitis pigmentosa (Senior Løken Syndrome) and discovered 2 new NPHP2/Inversin mutations and 2 families with NPHP5 mutations, respectively. The combined mutation analysis approach allowed us to rapidly identify rare mutations in a large number of patients.

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A Nonsense Mutation in the Corneodesmosin Gene in a Mexican Family with Hypotrichosis Simplex of the Scalp.

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Hypotrichosis simplex of the scalp (HSS; MIM 146520) is a rare autosomal dominant form of non-syndromic alopecia that affects men and women equally. Up to now, only a small number of families with HSS have been reported. The affected individuals experience a diffuse progressing hair loss from childhood to adulthood that is confined to the scalp. Recently, HSS has been mapped to the short arm of chromosome 6 (6p21.3), allowing mutations in the corneodesmosin gene (CDSN) to be identified as the cause of the disorder. To date, two stop mutations have been found in three unrelated families with HSS of different ethnic origin. Here, we describe a Mexican family presenting HSS. The pedigree extends over six generations comprising 156 individuals, 42 of whom are affected. By direct sequencing of the two exons of the CDSN gene, a novel nonsense mutation was identified in the index patient in exon 2, resulting in a premature stop codon (Y239X). The mutation co-segregated in the family (28 family members, 9 of whom were affected) and was not found in 300 control chromosomes using a restriction enzyme analysis with PsuI. Two additional, so far unknown SNPs in the coding region of the CDSN gene co-segregating in the family were found. Our data provide molecular genetic evidence for a 3rd stop mutation in exon 2 of the CDSN gene being responsible for HSS. All to date known nonsense mutations responsible for HSS are clustered in a region including 40 amino acids of exon 2 in the CDSN gene. Further families should be investigated to elucidate if this region of the CDSN gene might have a special effect on hair growth.

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Novel mutations of *ZFHXIB* responsible for the Mowat-Wilson syndrome. *Y. Yamada[1], K. Yamada[1], N. Nomura[1], N. Ishihara[1], Y. Kondo[1], K. Yokochi[2], T. Kuroda[3], A. Ota[4], N. Okamoto[5], M. Iwakoshi[6], J. Abe[7], S. Hamano[8], M. Saeki[3], M. Nagaya[9], N. Wakamatsu[1].* 1) Dept Genet, Inst Develop Res, Aichi Human Service Ctr, Aichi, Japan; 2) Seirei Mikatahara Hosp, Shizuoka, Japan; 3) Natl Ctr Child Health Develop, Tokyo, Japan; 4) Natl Kagawa Child Hosp, Kagawa, Japan; 5) Osaka Med Ctr Res Inst Maternal Child Health, Osaka, Japan; 6) Nishinomiya Municipal Wakaba-en, Hyogo, Japan; 7) Hyogo Pref Nishinomiya Hosp, Hyogo, Japan; 8) Saitama Child Med Ctr, Saitama, Japan; 9) Ctrl Hosp, Aichi Human Service Ctr, Aichi, Japan.

The mutations of *ZFHXIB* cause an autosomal dominant disorder of Mowat-Wilson syndrome associated with profound mental retardation, delayed motor development and specific facial features. We performed molecular analysis of *ZFHXIB* to confirm diagnosis of Mowat-Wilson syndrome. To screen for mutation, DNA fragments including each exon of *ZFHXIB* from patients and normal controls were amplified by PCR and subjected to direct sequencing analysis. Six novel mutations were found in the typical patients with Mowat-Wilson syndrome, one nonsense mutation (936C>A, C312X) and five frame-shift mutations including two mutations inserted one base (313insA, 105fs121X; 2254insA, 752fs754X) and three mutations deleted one base (1175delG, 392fs395X; 1535delG, 512fs515X; 1822delG, 608fs620X). We have identified 31mutations in *ZFHXIB* up to the present. In 29 typical cases with profound mental retardation and specific facial features, 14 nonsense mutations and 15 frame-shift mutations were detected. These mutations generated truncated protein without the zinc finger domain located in the C-terminal region.

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Novel Mutations and Variants Identified in the MECP2 Gene. *W. Sun[1], M. Peng[1], A. Buller[1], M. McGinniss[1], F. Quan[1], S. Potts[2], J. Taylor[3], C. Strom[1].* 1) Molec Gen Dept, Nichols Inst, Quest Diagnostics Inc, San Juan Capistra, CA; 2) Advanced Diagnostics Information Technology, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA 92690; 3) Quest Diagnostics, Inc., Wood Dale, IL 60191.

Rett syndrome (RTT) is one of the most common genetic causes of mental retardation in females. It is a neurodevelopmental disorder caused by mutations in the X-linked methyl CpG binding protein 2 (MECP2) gene. Direct sequence analysis of the MECP2 gene has been offered at Quest Diagnostics Nichols Institute to aide the diagnosis of RTT in affected individuals and to test for the potential presence of MECP2 mutations in patients parents. In more than 600 consecutive unique patients subjected to MECP2 mutation detection in our institute, 45 were found to have MECP2 mutations previously described to be associated with RTT. In addition, we report that we also detected 16 novel mutations and variants in the MECP2 gene. Four were frameshift mutations which are most likely to be pathogenic. There were also 5 missense changes located in the N-terminus, MBD, and the C-terminus domains. Interestingly, we detected 5 different in-frame deletions affecting the proline-rich region (aa. 384 to 393), a putative DNA-binding motif, in the C-terminus domain. The in-frame deletion of 2 to 6 amino acids in this region are likely polymorphic changes to the gene as 2 of the deletions were detected in both the symptomatic girls and their asymptomatic mothers. Proline-rich regions are commonly believed to adopt a secondary structure known as a left-handed polyproline helix (PPII). The polyproline region and other likely PPII regions in MeCP2 are variable in length in different orthologs, suggesting that the actual length of the PPII helices is less important in the interaction with DNA. We also detected two single nucleotide substitutions in the 3 untranslated region of the MECP2 gene. Our results shed new light on the genotype-phenotype relationship of the MECP2 mutations, and a better understanding of the MECP2 protein structure.

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Robot-assisted procedures prior to direct sequencing of the entire fibrillin 1 (*FBN1*) coding sequence as a strategy for the rapid mutation identification in patients with Marfan syndrome. *L. Tjeldhorn[1], S. Rand-Hendriksen[2], K. Gervin[1], K. Brandal[1], E. Inderhaug[1], O. Geiran[3, 4], B. Paus[1].* 1) Dept. of Medical Genetics, Ulleval University Hospital, Oslo, Norway; 2) TRS National Resource Centre for Rare Disorders, Sunnaas Rehabilitation Hospital, Nesoddtangen, Norway; 3) Dept. of Thoracic and Cardiovascular Surgery, Rikshospitalet University Hospital, Oslo, Norway; 4) University of Oslo, Norway.

The amplification and sequencing of the entire 235 kb gene, fibrillin 1 (*FBN1*), which is required for the identification of new mutations causing Marfan syndrome (MFS) and other type-1 fibrillinopathies, has been tedious. Most of the more than 600 known mutations in *FBN1* have been identified by indirect methods like SSCP, CSGE, and TGGE, screening for conformational changes prior to sequencing of selected fragments. However, it is not obvious that all mutations will be detected by screening for conformational changes. Optimizing conditions is time consuming and hence costly. Due to the size of the gene, new methods for high througput amplification and analysis is of particular interest to identify mutations in *FBN1*. We have been sequencing the entire *FBN1* coding sequence and flanking intronic sequences in 95 patients with suspected MFS, taking advantage of devices allowing for high throughput. The automated sequencing was preceeded by exonwise robot-assisted PCR setup, purification, and sequencing reactions. This allowed for a small reaction volume, reducing the costs. Using the programmed robot, preparation of PCR, purification, and preparation of sequencing reactions required less than one fifth of the time compared to manual workout. After sequencing all fragments once, mutations were identified in 62 individuals (65%). In the subgroup of patients fulfilling the diagnostic criteria for MFS (Ghent nosology) (n=76), mutations changing the amino acid sequence were identified in 57 individuals (75%), being in line with the mutation detection rate reported by others. We conclude that the robot-assisted method, making direct sequencing feasible as a primary strategy, is well suited for the rapid and accurate diagnostic mutation detection in the large gene, *FBN1*.

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Mutation analysis of *DNAI1* in large cohort of patients with Primary Ciliary Dyskinesia (PCD). *M. Zariwala[1], M.P. Kennedy[1], M.W. Leigh[1], P.G. Noone[1], J. Horvath[2], H. Omran[2], H.M. Mitchison[3], R. Chodhari[3], E.M.K. Chung[3], L.M. Morgan[4], R.U. De Iongh[4], J. Rutland[4], U. Pradal[5], M.R. Knowles[1].* 1) UNC-Chapel Hill, NC; 2) the Universitats-Kinderklinik, Freiburg; 3) Royal Free and University College Medical School, London; 4) Concord Hospital, Sydney; 5) Univeristy of Verona, Verona.

Primary Ciliary Dyskinesia (PCD) is characterized by sino-pulmonary disease and half the patients have situs inversus (Kartagener syndrome [KS]). This disease is inherited as an autosomal recessive trait and results from axonemal abnormalities; 80% have dynein arm defects in cilia. Pathogenic mutations have been identified in *DNAI1* and *DNAH5*. Without careful categorization of the PCD/KS patients, genetic heterogeneity is a significant limitation for large-scale genetic testing. As part of an overall effort to study the phenotype and genetics of PCD, we have studied a large number of PCD/KS patients, including detailed analyses of clinical features, nasal ciliary ultrastructure, and nasal nitric oxide measurement. The current study reports mutation analysis in this cohort of PCD for *DNAI1*, which is an intermediate chain dynein that encodes an outer dynein arm (ODA). There were 140 unrelated patients and ODA defects were noted in 65 and axonemal defects in the other 75. 13 families were excluded from linkage to *DNAI1* and remaining 127 patients were completely sequenced. A total of 11 patients (8%) harbored mutations and the rate of mutation detection was higher (12%) in patients with defined ODA defect. Of the 11 patients, 3 were homozygous for the previously identified 219+3insT mutation, 4 were compound heterozygous and 4 had monoallelic mutations. Taken together, the previously published and current studies show 219+3insT mutation in 16 of 30 known mutant alleles and analysis is underway to find a founder effect. Exon 17 is emerging as a mutation hot spot (6 of 30 known mutant alleles). In conclusion, we have detected that 12% of PCD patients with ODA defect harbor mutations in *DNAI1*, which will be useful in developing a clinical genetic assay for PCD. This abstract was funded by GCRC#00046, MO1 RR00046-42, 1 RO1 HL071798, 5 U54 RR019480.

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The Libyan Jewish mutation in dysferlin gene is common in Miyoshi myopathy patients from central Italy. *J. Mela[1,2], R. Di Giacopo[1], M. Catteruccia[1], R. Verardo[1], S. Servidei[1], G. Galluzzi[1,2].* 1) Neuroscience, Catholic University, Roma, Italy; 2) UILDM Rome-Section, Italy.

Miyoshi myopathy (MM), a distal muscular dystrophy, is caused by mutations in dysferlin gene on 2p13 that encompasses 55 exons and codes a 237 kDa protein localized to the plasma membrane of muscle fibers. Dysferlin function is not fully elucidated, but recently a central role in membrane-repair mechanisms was hypothesized. More than 100 pathogenic changes have been reported until now, but a common mutation (4872_4876delinsCCCC) was identified in the Libyan Jewish cluster with LGM2B/MM that was important for dysferlin gene characterization. We analyzed 23 Italian patients from 17 families with MM and reduction/absence of dysferlin protein on Western blot (WB) of muscle extracts. Onset affecting calf muscles varied from 14 to 20 years and progression was slow. Muscle biopsies showed dystrophic changes and marked inflammation. Genetic studies were performed by multiple heteroduplex analysis of all 55 exons and sequencing of DNA fragments with electrophoretic abnormalities. We found the mutation in 5 unrelated patients from non consanguineous families, all coming from a small area of central Italy around Rome. The mutation was absent in patients from the rest of Italy and beside the Libyan Jews cluster has been found in only one consanguineous French family. Four patients were homozygous and one was a compound heterozygote carrying in the second allele a new missense mutation in exon 51. The mutation causes a premature stop codon and accordingly dysferlin was absent on WB in homozygous patients, and reduced to 50% in a 14-year-old heterozygous asymptomatic carrier with high CK. In the compound heterozygote dysferlin was reduced to 10% of normal. Microsatellite analysis with polymorphic markers internal to dysferlin gene, showed identical haplotype associated with the mutation in all of the patients. The finding of 4872_4876delinsCCCC in two geographical clusters of populations strongly suggests the existence of a founder effect for this mutation and the importance of identifying common mutations in order to avoid time consuming genetic analyses in complex genes such dysferlin.

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Mutation analysis of the FGF14 gene in Portuguese patients with ataxia. *I.F. Bento[1], I. Alonso[1,2], P. Coutinho[3], J. Sequeiros[1,2], I. Silveira[1].* 1) UnIGENe, IBMC, Universidade do Porto, Porto, Portugal; 2) ICBAS, Universidade do Porto, Porto,Portugal; 3) Serviço de Neurologia, Hospital de São Sebastião, Feira, Portugal.

Spinocerebellar ataxias (SCAs) are a large group of neurodegenerative disorders with autossomal dominant inheritance and clinical overlapping, as well as a highly variable phenotype. Until now, 25 different loci have been identified, and 12 genes are associated with these diseases, showing a large genetic heterogeneity. Two different mutations were recently described in the fibroblast growth factor 14 gene (FGF14) in a Dutch family and in one German patient with SCA. These patients have a particular phenotype, presenting with slowly progressive cerebellar ataxia, early-onset tremor, memory loss, dyskinesia and mild mental deterioration. The gene FGF14 maps to chromosome 13q34 and encodes a member of a subclass of fibroblast growth factors involved in a variety of biological processes such as mitogenic and cell survival activities. FGF14 is expressed in the developing and adult central nervous system with functions in neuronal signaling, axonal trafficking and synaptosomal function. In order to determine the underlying mutation in a large group of Portuguese patients with ataxia, we performed mutation analysis of the FGF14 gene. We have thus analyzed 204 unrelated SCA patients of Portuguese origin, previously excluded for other known forms of ataxia. Clinical manifestations included progressive ataxia, associated or not with other symptoms, such as epilepsy, mental retardation or tremor. Molecular analysis of the FGF14 gene was performed by PCR amplification of the 5 exons, followed by SSCP and sequencing. Variants were detected in exons 1, 2, 4 and 5. Sequencing analysis of fragments with these variants is now being carried out and results will be presented.

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***De novo* G59V mutation in the *GJB2* (Connexin26) gene: a mutation hotspot for syndromic form of deafness and hyperkeratosis?** *R. Mao[1,2], B. Clifford[3], J. Palumbos[3], J. Metz[1], J.C. Carey[3].* 1) ARUP Laboratories; 2) Department of Pathology; 3) Department of Pediatrics, Division of Medical Genetics, University of Utah Health Sciences Center, Salt Lake City, Utah.

Connexin 26, encoded by the beta-2 gap junction protein (*GJB2*) gene, is one of the connexin proteins that play a major role in the gap junctions of cell-to-cell channels. It is expressed mainly in the brain, skin and cochlea. Mutations in GJB2 gene are the cause for more than 50% of profound pre-lingual genetic deafness. While most *GJB2* mutations are recessive, distinct dominant mutations in this gene are also demonstrated to underlie either skin disease or deafness or both. We present a *de novo* finding of G59V mutation in the *GJB2* gene in a 15-year-old girl having profound congenital hearing loss and hyperkeratosis on the palms. She was born after an uncomplicated pregnancy and family history of this condition is unremarkable. Sequencing analysis for the 5UTR and entire coding region of the *GJB2* gene for this patient showed a nucleotide substitution at position n.176 G>T, causing an amino acid alteration of Valine for Glycine at codon 59 (G59V). Both parents tested negative for this variant and misparentage was ruled out. In addition, the mutations of A1555G and A7445G in mitochondrial DNA tested negative in this individual. The G59V has not been previously reported. However, a G59A has been seen in a family with an autosomal dominant syndrome of palmoplantar hyperkeratosis and high-frequency hearing loss and a G59S was seen in a father and son with Bart-Pumphrey syndrome. Our patient has congenital hearing loss and similar skin findings as these two cases. Moreover, the Glycine59 is located within the highly conserved first extracellular loop of connexin 26, which is crucial for voltage gating and connexon-connexon interactions. Therefore, the G59V replacement may seriously compromise these functions. Our results demonstrate additional evidence that the G59 is one of the mutation hotspots in *GJB2* gene, associated with a dominant form of hearing loss and hyperkeratosis.

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Two novel mutations in Fz4 cause familial exudative vitreoretinopathy. *Z. Yang[1, 2], D. Shukla[3], K. Howes[1], E. Pearson[1], Y. Zhao[1], G. Karan[1], K. Zhang[1].* 1) Ophthalmology, University of Utah, Salt Lake City, UT; 2) Sichuan Medical Science Academy & Sichuan Provincial Peoples Hospital, China; 3) Anna Nagar, Madurai - 625 020, Tamilnadu, India.

Purpose: To describe two novel mutations in the Fz4 gene that result in familial exudative vitreoretinopathy. Methods: Ophthalmic examination and genetic study were conducted in two kindreds with familial exudative vitreoretinopathy (FEVR). Genomic DNA was extracted from blood samples of members of those kindreds. Each of the 2 exons of Fz4 gene was amplified by PCR and sequenced using ABI 3100 genetic analyzer. Wild-type and FEVR-causing mutants of hFz4 were assayed for hNorrin binding and hNorrin-dependent activation of classical Wnt pathway by cell surface binding assays and luciferase assays. Results: Of 8 individuals and 6 individuals who were at risk of inheriting the disease gene in two families with familial exudative vitreoretinopathy (FEVR), 5 and 3 were diagnosed with FEVR respectively based on decreased visual acuity and ophthalmic examination. DNA sequence analysis identified two novel missense mutations 133 G>A (C45Y) and 172 A>G (Y58C). This sequence changes segregated with the disease phenotype and were not observed in 200 normal controls. The C45Y and Y58C mutants are produced at wt levels in 293 HEK cells by immunoblotting, the two Fz4 CRD region mutations affected the Norrin-Fz4 binding and are severely defective in hNorrin-dependent classical Wnt signaling. Conclusions: We identified two novel mutations in FZ4 causing FEVR. This study identified a new type of Fz4 gene mutation in which the Fz4 CRD mutations affect the Norrin-Fz4 binding and consequent classical Wnt signaling defect.

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Screening of the dup(24bp) mutation of the *ARX* gene in mentally retarded patients with negative fragile X screening: First report in Thailand. *S. Rujirabanjerd[1], K. Tongsippunyoo[1], T. Sripo[1], J. Wirojanan[2], P. Limprasert[1].*
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The frequency of X-linked mental retardation (XLMR) is estimated to be 1 in 600 males. The most common disease is fragile X syndrome. Nevertheless, there are several genes which have been thought to cause XLMR. One of them is the Aristaless related homeobox *(ARX)* gene. Phenotypic effects of *ARX* mutations are varied depending on types and locations of the mutations in the gene. The most common mutation is an inframed-duplication of 24 bp in exon two resulting in an addition of 8 alanines to the second alanine tract in the protein. Here we report the first series of *ARX* mutation screening in Thai pediatric patients. The majority of samples were from southern Thai population, and ~40% were from hospitals in Bangkok. Genomic DNA from 192 mentally retarded patients was extracted from peripheral blood. All samples were initially screened for fragile X syndrome by PCR and Southern blot. None of them showed abnormal results. All samples were subsequently screened for dup(24bp) mutation in exon 2 of the *ARX* gene by PCR. Only one patient showed abnormal results. The duplication was confirmed by direct PCR sequencing. Clinical and DNA analysis were performed in all family members of the affected individual. The inheritance pattern of the affected family showed typical X-linking where the patient was confirmed to inherit a mutational allele from his mother. Clinical examination of three affected individuals in the family showed moderate to severe developmental delay. No history of seizure or infantile spasm was observed in any affected individuals. Dystonia, one of the most common clinical symptoms reported, or other abnormal movement was not detected at the time of examination. No other remarkable anomalies were seen. Considering pleiotropic effect in individuals with *ARX* mutation, the clinical variation found in this family was expected. Genetic counseling was subsequently provided to the first Thai family with *ARX* mutation.

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Characterization of hemophilia A and B mutations in Switzerland and a comparison of SSCA and direct sequencing approaches. *M.A. Morris[1], I. Surkova[1], A. Honsberger[1], P. de Moerloose[2], M. Schmugge[3], K. Peter-Salonen[4], E.O. Meili[5], G.A. Marbet[6], S. Hartmann[7], M. Neerman-Arbez[1], R. Kobelt[8].* 1) Service of Medical Genetics, University Hospital, Geneva, Switzerland; 2) Service of Angiology and Haemostasis, University Hospital, Geneva; 3) Department of Haematology, University Childrens Hospital, Zurich; 4) Central Haematology Laboratory, University Hospital, Bern; 5) Division of Haematology, University Hospital, Zurich; 6) Haemostasis Laboratory, University Hospital, Basle; 7) Medical Practice, Chur; 8) Swiss Hemophilia Association, Bern.

A nationwide program to characterize the mutations in all known hemophilia A and B patients was undertaken with the aim of providing better genetic services for families. The protocol was initially designed to provide good sensitivity of mutation detection at reasonable cost and then completed by direct sequencing of all F8C and F9 coding regions. Hemophilia A: 176 unrelated patients participated, 102 were defined as clinically severe. Patients were initially tested for the recurrent IVS22 inversions by Southern blotting. Inversions were found in 39 patients. The F8C gene was then screened by PCR-SSCA of all coding regions and exon-intron boundaries (for exon 14 only exon-intron boundaries). Exon 14 was also screened for nonsense mutations by PTT. All abnormal variants were sequenced. 39 patients with no identified mutations were screened by direct sequencing of all coding regions. Mutations were identified in 164/176 of all haemophilia A patients (93%), including 94% of severe, and 89% of mild and moderate patients. 102 discrete mutations were found, 54 of which have not been previously reported in the mutation databases. Hemophilia B: 39 unrelated patients participated in the study. The F9 gene was analysed by direct sequencing of the promoter region, all coding regions and exon-intron boundaries and the polyadenylation site. Mutations were identified in all patients but one (97%). We found 28 discrete mutations, of which 7 were novel. The results provide a national database of mutation data, the implications of which for phenotype-genotype correlations and genetic counselling will be discussed.

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Screening for Novel Mutations in the Coding Region of the Ryanodine Receptor in Individuals with Malignant Hyperthermia Susceptibility. *S. Levano, M. Singer, A. Urwyler, Th. Girard.* Departments of Anesthesia and Research, University Hospital, Basel, Switzerland.

Malignant hyperthermia (MH) is a dominantly inherited pharmacogenetic disorder of skeletal muscle. MH is triggered by inhalative anesthetics in genetically predisposed individuals and the ryanodine receptor 1 (RYR1) has been identified as it's primary locus. In our MH center 40 % of Swiss families have shown causative mutations after sequencing the three hot spots of the RYR1 gene. We decided to analyse the entire coding region of RYR1 in the remaining Swiss families with unidentified mutations in order to increase the number of mutations eventually used for molecular genetic testing in MH. We selected 36 unrelated individuals from Swiss MH families, with ? 0.5g contracture following 2% halothane and 2mM caffeine in the in vitro contracture tests (IVCT). The RYR1 transcript was amplified by polymerase chain reaction (PCR) and analysed by direct-sequencing. Restriction fragment length polymorphism method was used within families with identified mutations in order to further investigate this mutation. Our mutation detection rate was 70% (26/36). Of the identified mutations 5 have previously been reported and 22 are novel. Eight individuals carried two different mutations, while two individuals had three mutations in the RYR1 transcript. Two novel mutations, R2336H and D2730G, were examined in detail. The mutation R2336H was detected in 7 families, and D2730G in one family. Results of the genetic analysis for both mutations showed good correlation with IVCT results. Out of 21 MH susceptible (MHS) patients evaluated for R2336H, 17 were mutation carriers and all 17 MHS in the family with mutation D2730G were mutation carriers. All MH normal patients were negative for both mutations. In a preliminary screening of 20 normal control chromosomes both mutations were not found. Analysis of the RYR1 transcript by PCR and sequencing showed a high detection rate of RYR1 mutations. In further steps we will investigate the functional effect of the novel mutations and will hopefully be able to offer molecular genetic diagnosis of MH susceptibility to a higher number of Swiss MH families.

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LRRK2 G2019S mutation is common in North-African Parkinsons disease. *S. Lesage[1], L. Hafid[1], P. Ibanez[1], E. Lohmann[1, 3], A. Dürr[1, 2, 3], A. Brice[1, 2, 3, 4].* 1) INSERM U679 (formerly U289), Neurologie et Thérapeutique Expérimentale, Hôpital de la Pitié-Salpêtrière, AP-HP, 75013 Paris, France; 2) Département de Génétique, Cytogénétique et Embryologie, Hôpital de la Pitié-Salpêtrière, AP-HP, 75013 Paris, France; 3) Fédération de Neurologie, CHU Pitié-Salpêtrière, AP-HP, 75013 Paris, France; 4) UFR Pierre et Marie Curie, AP-HP, 75013 Paris, France.

Background : Mutations in the LRRK2 gene at the PARK8 locus have recently been identified in Parkinsons disease (PD), the second most frequent neurodegenerative disease. Preliminary studies from us and others indicate that the most common LRRK2 G2019S mutation is implicated in 3-6% and 1-2% of autosomal dominant PD and apparently sporadic forms of PD, respectively. Aim : To determine the relative frequency of the LRRK2 G2019S mutation in a sample of familial and sporadic PD index cases from North-African origin and to investigate for a shared haplotype in the G2019S mutation carriers. Patients and methods : Our North-African sample included 68 PD index cases (24 familial cases and 44 sporadic cases) and 48 ethnically matched controls. Exon 41 of the LRRK2 gene was screened by dHPLC and direct sequencing. Shared haplotype by LRRK2 G2019S was estimated by typing microsatellites and SNPs located within and in the vicinity of the LRRK2 gene. Haplotypes were constructed using the Merlin program. Results : The LRRK2 G2019S mutation was identified in 22 index cases including 2 homozygotes resulting from consanguineous families. Of them, 11 were familial cases (11/24, 46%) and 11 were sporadic cases (11/44, 25%). No mutation was found in controls. By analysing microsatellites and SNPs, we found a 60 Kb shared haplotype. Genotype/phenotype correlation analyses are in progress. Conclusion : Our results suggest that the LRRK2 G2019S mutation is highly common in North-African Parkinsons disease with an overall frequency of 32%. In addition, the G2019S carriers from North Africa shared the same common haplotype than PD patients from European origin, suggestive of a founder effect.

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MEDICAL-GENETIC COUNSELING OF THE NERVOUS SYSTEM HEREDITARY DISORDERS IN MOLDOVA. *V.C. Sacara.* Medical Genetics, NCRHMGFP, Chisinau, Moldova, Republic of.

The first population genetic study of hereditary disorders of the nervous system (HDNS) in Republic of Moldova was carried out. A total of 19 nozological forms were revealed, included ten autosomal dominant (43 families with 80 affected individuals), six autosomal recessive (95 families with 99 affected) and two X-linked (106 families with 117 affected) diseases. Pooled prevalence of HDNS in Moldova didnt correspond to the average prevalence for other populations, for example Russian. Substantial differences between urban and rural populations were not observed. The composition of the HDNS spectrum nucleus in Moldova displayed number of difference from that in the majority of other examined. DNA analysis of the DMD/B families consists of detects deletions in 13 different exons of dystrophin gene and RFLP-analysis (pERT87-8/TaqI, pERT87-15/BamH1 and 16intron/TaqI), the algorithm of DMD/B molecular researches to define informative in 93%. The molecular studies at the SMNI locus (SMA disease) were performed. Homozygous deletions of the 7 and/or 8 exons were in 91%.. Some genetics features of the hereditary motor-sensory neuropathies have been revealed (intrafamilial polymorphism of different degree, effect of the ancestor in the dominant forms). According to publication date most CMT1A cases (>98%) are caused by a duplication of a 1.5 Mb region on the chromosome 17 containing the PMP22 gene. We test 2 STRs located within the duplication (tetra- and pentanucleotide repeats) 54 DNA samples of unrelated CMT1 patients . The CMT1A duplication was determine in 24.07% cases (gene dosage for heterozygous samples- different fluorescent intensity and /or three alleles). The presence of two alleles (59.3%) was used to indicate that no duplication was present in samples. Homozygous samples 16.6% required a competitive gene dosage test. The data obtained may constitute a basis for regional registration of HDNS in Moldova.

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New CFTR gene variants in Cystic Fibrosis and Chronic Rhinosinusitis Chilean patients. *G. Molina[1], C. Musri[1], M. González[1], A. Vera[1], V. Lezana[2], A. Milinarsky[2], F. Krause[3], FJ. González[1].* 1) Laboratorio de Biología y Genética Molecular. Escuela de Medicina. Universidad de Valparaíso. Valparaíso. Chile; 2) Departamento de Pediatría. Escuela de Medicina. Universidad de Valparaíso. Valparaíso. Chile; 3) Cátedra de Otorrinolaringología. Escuela de Medicina. Universidad de Valparaíso. Valparaíso. Chile.

Cystic Fibrosis (CF) is the most prevalent autosomal recessive disease in the Caucasian population (1/2000 newborns). Its prevalence in Chile could vary between 1/4000 to 1/2000 newborns in different regions. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a protein that functions as a chloride channel. This protein regulates electrolytes and water transport through secretory cells. The failure of this protein produces a greater viscosity of mucus and other secretions. Chronic Rhinosinositis (RC) is a CFTR related disease, since greater viscosity of mucus is one of its causes. More than 1000 CFTR gene mutations have been described. We have searched for new mutations in 20 CF patients, using SSCA and sequencing of exon4, exon7, exon10 and exon17b. We have already found two variants(p.T351S, p.G528G) in three chromosomes. We have not detected the p.T351S variant in 100 chromosomes of individuals from Valparaisos general population, thus it could be a CF causative mutation. Conversely, p.G528G is a common polymorphism. Furthermore, we have determined that homozygous individuals for this variant have an exon 10 normal splicing, despite the fact that it modifies the last base of exon 10. In RC patients we have performed a complete screening of CFTR coding sequencing using RT-PCR of peripheral lymphocytes RNA. In one patient, we have found a splicing variant causing exon 5 skipping. This approach could lead us to discover new mutations responsible for the unknown 50% of CF causative mutations in our CF patients and to clarify the relationship between CF and RC. DIPUV 39/2004.

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Variants in Tissue Necrosis Factor Increase Survival While Variants in Mannose Binding Lectin 2 Decrease Survival in Cystic Fibrosis. *K. Buranawuti[1], M. Boyle[2], G. Cutting[1].* 1) Inst of Genetic Medicine; 2) Dept of Pulmonary Medicine, Johns Hopkins School of Medicine.

Cystic Fibrosis (CF) patients with the same mutations in the CF Transmembrane Conductance Regulator (CFTR) gene can differ widely in survival suggesting other factors play a substantial role in mortality. Since dysregulated inflammation is believed to play a major role in CF pathogenesis, we determined whether variation in 36 genes (51 single nucleotide polymorphisms; SNPs) involved in innate immunity and inflammatory response influence survival of CF patients. The 51 SNPs were typed in 4 Caucasian groups recruited from the same medical center: 115 CF survivors (patients > 16 y.o.), 101 CF children (16 y.o.), 176 disease controls (chronic sinusitis patients), and 127 normal controls. Three comparisons of genotype frequencies were performed using Monte-Carlo permutation analysis; CF survivors vs. CF children, CF survivors vs. disease controls and CF survivors vs. normal controls. Five SNPs demonstrated unequal genotype frequencies in two of the three comparisons (IL1, IL5R, IL6, IL13, and TGF1) and two SNPs were unequal in all three comparisons (TNF and MBL2). The genotype frequencies of these seven SNPs were then compared between CF survivors and 38 CF patients from the same birth cohort and same medical center who had died (21) or had lung transplantation (17) after 16 years of age. Single SNPs from only two of the seven genes displayed significant differences in genotype frequencies: TNF-238 G/A genotype ($p<0.001$) and MBL2 O/O genotype ($p< 0.009$; Fishers exact). Kaplan-Meier plots of age at death revealed that survival of patients bearing TNF-238 G/A was increased significantly vs. G/G ($p= 0.02$) and survival of patients bearing MBL2 O/O was decreased significantly vs. A/A or A/O ($p =0.004$; Log Rank P value). These effects were independent of CFTR genotype. The logistic regression analysis indicated that the odds ratio for survival is 9 times greater for CF patients with the TNF-238 G/A while the odds ratio for survival of the MBL2 O/O is 8 fold lower. Thus, TNF-238 G/A and MBL2 O/O appear to be positive and negative modifiers of CF survival, respectively.

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Alternative splicing analysis of *Pkhd1*, the ARPKD gene, in kidney and liver. *R. Punyashthiti, C.J. Ward, S.L. Whelan, J.R. Woollard, V.E. Torres, P.C. Harris.* Nephrology, Mayo Clinic College of Medicin, Rochester, MN.

Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations to *PKHD1*, a large multi-exon gene on chromosome 6. The *PKHD1* protein product, fibrocystin, is predicted to be a large glycosylated transmembrane protein with a short cytoplasmic tail. Northern and RT-PCR data has let to the proposal that *PKHD1* has multiple differentially spliced transcripts that give rise to several protein isoforms, including secreted products.

Here we describe analysis of *Pkhd1* variants by northern blotting, RT-PCR, and 5RACE in mouse to assess the full level of variability. A major 13kb mRNA transcript was detected by northern blotting on adult kidney by several probes with additional faint products of 9 and 7.5kb detected by probes to the 5 part of the gene. Long-range RT-PCR was also consistent with a modest level of variability with a single product generally detected, but with a number of smaller variants in the exon 52 to 62 region. One of the products was found to have 961 nt of exon 61 missing, and generate a premature stop codon before nucleotides encoding the transmembrane domain. This mRNA could encode a secreted fibrocystin isoform lacking the cytoplasmic tail. This splice form is found in kidney and biliary tree, suggesting functional significance.

At the other end of the gene, we have sought novel mRNAs by 5RACE that may explain the lack of/ mild kidney phenotype in a mouse model with the first coding exon (exon 2) removed. The major detected transcript was that predicted, splicing from exon 1 to 3, but a variant that has lost exon 5, and is predicted to utilize a novel start codon in exon 4, when exon 2 is missing, was also found. However, this product does not have a classical signal peptide and does not seem to function as an efficient signal peptide in an in vitro assay.

Our studies suggest that *Pkhd1* does not generate countless fibrocystin molecules, but that specific splice variants are generated in kidney and liver and that some specific variants may be critical for normal function.

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APTX, spinocerebellar ataxia protein, is an exonuclease and releases blocked DNA 3-ends. *M. Tada[1], T. Takahashi[1], S. Igarashi[1, 2], A. Yokoseki[1], H. Date[3], H. Takano[1], M. Oyake[1], S. Tsuji[3], M. Nishizawa[1], O. Onodera[1, 2].*
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Purpose: To investigate the function of aprataxin (APTX) on DNA substrates. Background: APTX is the causative gene product for the early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH/AOA1), a spinocerebellar ataxia with peripheral axonal neuropathy. APTX binds to X-ray repair cross-complementing group 1 protein, which is the scaffold protein for base excision repair machinery in SSBR. The lymphoblastoid cells of EAOH/AOA1 patients are sensitive to agents which cause single strand breaks. These findings support the idea that APTX plays an important role in SSBR. The roles of APTX in SSBR, however, remains unclear. Materials & Methods: His-tagged human full length APTX was expressed using baculovirus expression system and was purified to homogeneity by metal affinity and gel filtration chromatography. Nicked double strand or single strand DNA substrates labeled by fluorescein were incubated with APTX protein at 37C. The products were analyzed by electrophoresis in a 20% polyacrylamide denaturing gel. Results: APTX had bidirectional exonuclease activities. Although some 3'5' exonucleases preferentially excise a mismatched DNA 3'-terminus to increase fidelity of DNA repair system, the APTX equally excised matched and mismatched DNA termini. Furthermore, APTX hydrolyzed the phosphate and phosphoglycolate at 3'-end. Conclusions: We showed that the APTX had the bidirectional exonuclease, 3'-phosphatase and 3'-phosphoglycolate hydrolase activities. These findings suggest that APTX removes blocked 3'-ends which interfere SSBR. Spinocerebellar ataxia with axonal neuropathy-1 is caused by mutation of tyrosil DNA phosphodiesterase 1 (TDP1), which also removes a tyrosyl-modified 3'-end. The mechanism of neurodegeneration by impairment of removing blocked 3'-end should be elucidated.

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Identification of a functional nuclear localization signal in the human -dystroglycan. *M. Bermudez de Leon[1], L. Fuentes-Mera[1], P. Gomez[1], V. Tapia-Ramirez[1], F. Garcia-Sierra[2], B. Cisneros[1].* 1) Dept. Genetics & Mol. Biol; 2) Dept. Cell Biol., CINVESTAV-IPN, Mexico city, Mexico.

The dystroglycanopathies are a novel group of human muscular dystrophies caused by gene mutations in essential transmembrane adhesion receptors. -dystroglycan is an extracellular protein that interacts with both; laminin at the extracellular matrix and -dystroglycan (DG) at the cellular membrane. In turns, DG is a transmembranal protein that interacts with dystrophin and actin via its cytoplasmic domains. Recently, we have observed that DG is present in the nucleus of HeLa cells suggesting that this protein could have a nuclear function. To determine the cellular signals controlling the nuclear localization of DG, we fused its cDNA to that of the green fluorescent protein (GFP). We found, by immunofluorescence and confocal microscopy analyses that the GFP-DG protein fusion immunostaining localized primarily in the cytoplasm of HeLa cells showing also a punctuate pattern in nucleus, which was excluded from the nucleoli. Such subcellular localization pattern was similar to that of the endogenous DG. We identified a stretch of basic amino acids in the DG C-terminal domain that could correspond to a nuclear localization sequence (NLS). Interestingly, the removal of the putative NLS prevented the nuclear localization of the GFP-DG fusion protein and caused an abnormal cytoplasmic accumulation. Currently, by site-directed mutagenesis, identification of the essential amino acids in the NLS is underway. Finally, the presence of a potential nuclear exporting signal was evaluated by leptomycin B-treatment. No alterations in the DG subcellular localization were observed in the leptomycin B-treated HeLa cells. In conclusion, the DG contains a functional NLS that regulates its subcellular distribution and might modulate its cellular functions.

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Axonal swellings and impaired axonal transport in a mouse model of spastic paraplegia linked to spastin mutation. *C. Fassier[1,4], A. Tarrade[1,4], S. Courageot[1], D. Charvin[1], A. Thorel[2], N. Roblot[1], A. Dierich[3], J. Melki[1].* 1) Dept Molecular Neurogenetics, INSERM E-223, Evry, France; 2) Centre des Matériaux, Evry; 3) IGBMC, Illkirch; 4) equal contribution.

Hereditary spastic paraplegia (HSP) is a neurological disorder characterized by axonal degeneration of corticospinal tracts. The most prevalent form of autosomal dominant HSP is linked to spastin (Sp) mutations, a microtubule severing protein. To elucidate the pathophysiology of HSP, homologous recombination in murine ES cells has been used to create a deletion of Sp exons 5 to 7. Mice carrying heterozygous (Sp/+) or homozygous (Sp/) deletion have been generated and characterized. Deletion of Sp was confirmed at the genomic, transcript and protein levels. Transverse semi-thin sections of spinal cord revealed axonal swellings in mutant but not control mice. The frequency of axonal swellings was increasing with aging. In addition, they were more frequent in (Sp/) than in (Sp/+) mutant mice of the same age suggesting a haploinsufficiency mechanism. No abnormality of sciatic nerve axons was observed. Spinal cords were then processed for electron microscopic analysis. Axonal swellings were filled with mitochondria, multilamellar bodies, lysosomes, membrane dense bodies and neurofilaments which strongly suggest an axonal transport defect in vivo. Interestingly, primary culture of cortical neurons derived from 14 d.p.c. embryos revealed neurite swellings in mutant but not control cells, features in agreement with in vivo data. The number of neurite swellings was higher in (Sp/) than in (Sp/+) cells, suggesting once again a haploinsufficiency mechanism underlying HSP linked to Sp mutation. Moreover, the lack of glial cells in our neuron culture indicated that Sp acts cell autonomously in neurons by contributing to neurite integrity. Our results show for the first time that Sp mutations are responsible for a progressive axonopathy restricted to the central nervous system and characterized by axonal swellings and axonal transport defect. Such results provide strong evidence that microtubule severing activity is an essential process for maintaining axonal integrity and transport in vivo.

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Transgenic strategy to determine new sites of WNK1 expression, a gene responsible for FHHt. *C. Delaloy, M. Clemessy, J. Hadchouel, X. Jeunemaitre.* INSERM, Collège de France, Paris, France.

WNK1 and WNK4, two genes belonging to a new serine-threonine kinase family are responsible for FHHt, an autosomal dominant form of hypertension. The biological and clinical features of the syndrome have highlighted the importance of their expression in the renal tubule and their role in the regulation of ionic transport. However WNK1 gives rise to several isoforms, some ubiquitously expressed with a complete kinase domain (LI: Long Isoforms) and one specifically expressed in the distal nephron wich is kinase defective arising from alternate promoter. Expression of both isoforms may be affected in FHHt patients bearing large deletions of WNK1 first intron. To understand the mechanism of the pathology and the role of WNK1 in blood pressure regulation we characterized the expression of WNK1 isoforms by using a transgenic approach. The nlacZ reporter gene was inserted in a BAC transgene that includes 160kb of the mouse WNK1 locus. Three founder lines from this construct showed ubiquitous LacZ staining which recapitulated the expression pattern seen by northern blot in different tissues and by immunostaining in polarized epithelia. We analysed in more detailed the LI-WNK1 expression in embryos of various stages and in adult organs and compared the results to those of in situ and in toto hybridizations. LI-WNK1 expression was detected in the early developing cardiovascular system. In adults positive staining was observed in arteries and veins, in endothelial cells as well as smooth smooth muscle cells. Whereas a diffuse and weak expression in the kidney was expected we observed an expression mainly restricted to the CTAL and DCT. In the central nervous system the transgene was expressed specifically in the cerebellum. These results suggest that WNK1 may be involved in the morphogenesis of cardiovascular development and cardiovascular function in adult. Together with the lethality of LI-WNK1 deficient embryos and the low blood pressure observed in heterozygous mice without any metabolic disorder, they also suggest that a perturbation of the LI-WNK1 expression might play a role in the increased BP observed in FHHt.

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Over-expression of Syntaxin 1A in mice results in abnormal glucose homeostasis similar to that seen in patients with Williams-Beuren syndrome. *P.P.L. Lam[1,2], Y.M. Leung[1,2], L. Sheu[1,2], J. Ellis[3], R.G. Tsushima[1,2], H.Y. Gaisano[1,2], L.R. Osborne[1].* 1) Medicine, University of Toronto, Toronto, Ontario, Canada; 2) Physiology, University of Toronto, Toronto, Ontario, Canada; 3) Developmental Biology, Sick Kids Hospital, Toronto, Ontario, Canada.

The SNARE protein Syntaxin 1A (STX1A) plays an essential role in exocytosis, and also binds and regulates $Ca2^{+}$ and K^{+} channels to influence the sequence of events leading to insulin secretion. Pancreatic islet levels of STX1A are reduced in type-2 diabetic rodents, but normal glycemic function can be restored by adenoviral transfer of STX1A. Patients with Williams-Beuren syndrome (WBS), who harbor a 1.5 Mb deletion on chromosome 7 encompassing the STX1A gene, also exhibit a variety of endocrine phenotypes, including an increased incidence of diabetes mellitus and a high frequency of impaired glucose tolerance. Taken together, these studies suggest that fluctuating levels of STX1A in pancreatic islets have an exquisite control over the different components of insulin secretion. We investigated the specific role of STX1A in pancreatic -cells by generating transgenic mice with a 30% increase in STX1A protein in pancreatic islets. The STX1A mice displayed fasting hyperglycemia and a more sustained elevation of plasma glucose levels after an intraperitoneal glucose tolerance test, with correspondingly reduced plasma insulin levels. Surprisingly, -cells from the STX1A male mice also exhibited abnormal insulin tolerance. We employed single cell analyses of exocytosis by patch clamp membrane capacitance (Cm) measurements and ion channel recordings to determine the -cell secretory defects. Depolarization-evoked Cm increases were reduced in the STX1A mouse islet -cells and the mice also exhibited reduced currents through $Ca2^{+}$ channels. These results suggest that even moderate changes in the level of STX1A protein can have a dramatic influences on the differential regulation of -cell ion channels and the exocytotic machinery. We hypothesize that the impaired glucose homeostasis seen in patients with WBS is a direct effect of the deletion of one copy of the STX1A gene, causing a change in STX1A protein levels in -cells.

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Developmental Pathways Involved in Synostosis of Coronal Sutures in Twist1 Heterozygous-Null Mice. *Y. Wang, J. Cai, E.W. Jabs.* Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Saethre-Chotzen syndrome is an autosomal dominant condition with both craniosynostosis of the coronal sutures and limb abnormalities. Most Saethre-Chotzen patients have loss of function mutations in TWIST1, a basic helix-loop-helix transcription factor critical in craniofacial and limb development. Some patients have a FGFR3 P250R mutation. TWIST1 has been implicated in the inhibition of differentiation of multiple cell lineages, including muscle and bone, but the molecular signaling pathways related to TWIST1 are poorly understood. The DNA binding sequences for TWIST1 are not well known either. There is some evidence in mouse and Drosophila that Twist1 and Fgfrs may be components of the same molecular pathway. Twist1 heterozygous-null mice have been created and have variable expression of craniofacial and limb defects consistent with Saethre-Chotzen syndrome. To investigate the downstream targets of TWIST1 signaling pathways involved in craniosynostosis, we dissected developing coronal sutures from heterozygous-null Twist1 mice as well as normal littermates, and performed microarray gene expression analysis on sutural RNA samples. We were able to identify 33 and 203 genes differentially expressed (p0.05) in coronal sutures between mutant and wildtype mice, at E16 and E18 stages, respectively. Interestingly, one of these genes expressed at E18 is Spry1, which is known to be induced by activating mutations in Fgfr3. Only three genes (Tac1, Osmr and 1110017D07Rik) as well as Twist1, were differentially expressed at both stages. These differentially expressed genes are overrepresented from functional categories of morphogenesis, organogenesis, and bone mineralization/remodeling. Some of these genes may be directly controlled by Twist1, and may share common promoter sequences that would be overrepresented in these genes. The identification of downstream targets of Twist1 could provide valuable information about additional factors that may be important in Twist1 and Fgfr3 signaling pathways and the pathogenesis of Saethre-Chotzen syndrome.

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Mouse Model for Apert Syndrome Fgfr2 +/P253R. *M. Sun, Y. Wang, R. Xiao, D. Huso, E.W. Jabs.* Johns Hopkins Univ, Baltimore, MD.

Apert syndrome (OMIM 101200), an autosomal dominant disorder characterized by severe skull malformations and syndactyly of the hands and feet, is caused by gain-of-function mutations in fibroblast growth factor receptor 2 (FGFR2). Approximately 33% of Apert syndrome patients have a FGFR2 P253R mutation while the others have a FGFR2 S252W mutation. It has been hypothesized that the P253R mutation is associated with milder craniofacial abnormalities and more severe syndactyly than the S252W mutation. Our laboratory previously created and characterized a mouse model for the S252W mutation (Wang et al. Development, in press). The generation of a knock-in mouse model with the P253R mutation will be very useful to study phenotype-genotype correlations between the two Apert syndrome FGFR2 mutations and the pathogenesis of this condition.

We engineered an Fgfr2 +/P253R knock-in mouse model. Necropsy, histological analysis, and bone and cartilage staining were conducted on heterozygous mutant mice at postnatal day 0 (P0), P5 and P10. Many abnormalities of the skeleton, including skull, long bone and sternum were found. An incompletely fused palate was present in all the mutant mice from embryonic 16.5 (E16.5) to P0. Abnormal cartilage at the midline sagittal suture, the coronal suture and long bones were noted. Presynostosis/synostosis was found at the mutant coronal suture. Abnormal proliferation and differentiation were found at the midline sagittal suture. There was no apparent difference in apoptosis between mutants and control littermates at the sagittal suture at P0. None of the mutants exhibited syndactyly. These observations are similar to those found in the Fgfr2 +/S252W mice except that the Fgfr2 +/P253R mice can survive longer and may have milder phenotypes. Both Fgfr2 mutations result in abnormal cartilage as well as bone formation which may give insight into the molecular process of cell fate determination of the chondrocyte and osteoblast lineages. [This work has been supported by the International Collaborative Genetics Research Training Program (NIH D43 TW 06176)].

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Mouse Model of Adenylosuccinate Lyase Deficiency. *E.K. Spiegel[1], S. Forbes[2], D. Patterson[2].* 1) Human Medical Genetics, University of Colorado Health Sciences Center, Denver, CO; 2) Eleanor Roosevelt Institute, University of Denver, Denver, CO.

Adenylosuccinate lyase deficiency is a disease of purine metabolism which affects patients both biochemically and behaviorally. The symptoms include psychomotor retardation, autistic features, hypotonia, and seizures. Patients also accumulate the substrates of ADSL in body fluids. Both the presence of normal levels of ADSL enzyme activities in some patient tissues and the absence of a clear correlation between mutations, biochemistry, and behavior show that the system has unexplored biochemical and/or genetic complexity. It is unclear whether the pathological mechanisms of this disease result from a deficiency of purines, a toxicity of intermediates, or perturbation of another pathway or system. Our goal is to produce a mouse model of this disease using mutations found in patients, which will aid in elucidating the *in vivo* mechanisms of this disease. A patient with autistic features and mild psychomotor delay carries two novel mutations in this gene, E80D and D87E. Using site-directed mutagenesis, we have engineered these mutations and the most common ADSL mutation, R426H, in a human genomic ADSL P1 clone. Transgenic mice have been created with these constructs and with the wild type human ADSL gene. We have also used mouse ES cells with an inactivated ADSL gene to introduce this ADSL knockout mutation into mice. The creation of a mouse model of ADSL deficiency will be accomplished by crossing lines with mutated human ADSL transgenes to the knockout ADSL line. These ADSL deficient mice will be analyzed biochemically for levels of purines and intermediates, and for enzyme activity. These mice, humanized for ADSL mutations, will allow us to investigate the roles of purine deficiency and intermediate accumulation in ADSL pathology, to test for behavioral changes resulting from specific ADSL mutations, and to test therapies to reverse the biochemical deficits in the mutant mice.

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A2E Accumulation Associated with Mutant *ELOVL4* Expression in Mice. *J. Cameron[1,2], G. Karan[1,2], Y. Chen[1,2], Z. Yang[1,2], Y. Zhao[1,2], S. Kamayama[1,2], A. Azimi[1,2], E. Pearson[1,2], H.R. Vollmer-Snarr[3], K. Zhang[1,2].* 1) Department of Ophthalmology and Visual Sciences, University of Utah, Salt Lake City, UT; 2) Program in Human Molecular Biology and Genetics, University of Utah, Salt Lake City, UT; 3) Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT.

The gene *ELOVL4* causes dominant Stargardt-like macular dystrophy (STGD), a juvenile onset macular degeneration that shares many features with age-related macular degeneration (AMD), including lipofuscin accumulation in retinal pigment epithelial cells (RPE) and degeneration of photoreceptors. Recently we have shown that transgenic mice expressing the mutant human *ELOVL4* are a good model for both STGD and AMD. These mice have significant declines in their retinal electrophysiological responses, as well as, substantial photoreceptor degeneration and lipofuscin accumulation. Here we further demonstrate that A2E, a major component in lipofuscin, increases in a dynamic fashion with *ELOVL4* expression and severity of RPE degeneration.

Using HPLC and MS to analyze RPE homogenates from *ELOVL4* transgenic mice we see an increase in A2E levels associated with a moderate to high expression of *ELOVL4* and a moderate loss of photoreceptors. However, allowing photoreceptor degeneration to become more severe in the moderate and high *ELOVL4* expressing mice results in a significant decrease in A2E levels. Such a decrease can be attributed to the lack of photoreceptors and RPE atrophy in the central retina. This data suggests that A2E formation varies due to *ELOVL4* expression and severity of degeneration in the central RPE. *ELOVL4* mice provide a valuable model for therapeutic trial of macular degeneration.

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Epistatic interaction between fibrillin-1 and effectors of TGF signaling in the pathogenesis of aortic aneurysm in Marfan syndrome. *T.M. Holm[1], B. Loeys[1], J. Habashi[1], L. Meyers[1], D. Bedja[1], E. Neptune[1], D. Judge[1], H. Dietz[1,2].* 1) Institute for Genetic Medicine; 2) HHMI, Johns Hopkins Univ., Baltimore, MD.

Marfan Syndrome (MFS) is a connective tissue disorder caused by mutations in the fibrillin-1 gene (FBN1). Clinical manifestations include ectopia lentis, aortic dilatation and dissection, and long bone overgrowth. Previous studies of Fbn1-targeted mice demonstrated that failure of distal lung septation and myxomatous change of mitral valves are correlated with excessive TGF activation and were rescued by TGF antagonism in vivo. We hypothesized that decreasing the expression of Smad4, a positive effector of TGF signaling, would rescue phenotypes due to excessive TGF activation. Smad4-haploinsufficient mice were crossed to Fbn1-targeted mice. Surprisingly, compound mutant mice die at six times the rate of mice carrying the Fbn1 mutation alone. Furthermore, echocardiograms showed large aneurysms above the sinotubular junction that were unique to compound-mutant mice. Vascular disease was absent or limited to the root of the aorta in the single mutants (Smad4+/- and Fbn1+/-, respectively). As opposed to the Fbn1+/- mice, which show wall thickening and excess collagen deposition, compound mutants showed thinning and collagen paucity. Nuclear pSmad2 (marker of TGF signaling) was enriched in Fbn1+/- mice compared to controls, but greatly reduced in compound mutants. It seemed possible that excessive TGF signaling induced by fbn-1 deficiency might limit signaling through other Smad4-dependent cascades (eg BMPs); this effect would be amplified in the Smad4-deficient state. In support of this view, we found that TGF neutralizing antibody attenuates aortic dilatation in Fbn1+/- mice, while prenatal BMP antagonism by conditional overexpression of Smad6 induces synthetic lethality. Curiously, Smad4 haploinsufficiency rescues the TGF-induced failure of lung septation in fibrillin-1 deficient mice. These data suggest tissue-specific differences in the secondary effects attributable to excess TGF signaling. While the complexity of crosstalk between TGF signaling cascades requires further elucidation, these data will inform the effort to identify genetic modifiers of MFS in humans.

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Mutant Wnk4 reveals mechanisms of hypertension and hyperkalemia. *M.D. Lalioti[1], J. Zhang[2], K.T. Kahle[1], K. Hoffmann[1], H.R. Toka[1], C. Nelson-Williams[1], C.J. Booth[3], Y. Lu[1], D.S. Geller[2], R.P. Lifton[1,2,4].* 1) Depts of Genetics; 2) Nephrology; 3) Section of comparative Medicine, Yale Univ Sch Medicine, New Haven, CT; 4) Howard Hughes Medical Institute.

Point mutations in the serine-threonine kinase gene *Wnk4* cause pseudohypoaldosteronism type II (PHAII), an autosomal dominant disorder featuring hypertension, hyperkalemia, and metabolic acidosis. *In vitro* studies have shown that wild-type (WT) WNK4 inhibits the NaCl cotransporter NCCT and the K+ channel ROMK and that PHAII mutations have divergent effects: inhibition of NCCT is lost and inhibition of ROMK increases. To extend these observations *in vivo*, we used a bacterial artificial chromosome (BAC) containing the mouse *Wnk4* and introduced the PHAII-specific Q562E mutation by recA-assisted recombination. We have also engineered a control BAC carrying a single nucleotide polymorphism (SNP) in the Wnk4 gene. In animals carrying two copies of the transgene, its tissue distribution and expression was virtually identical to the endogenous gene. Compared to WT littermates, PHAII mice displayed significantly elevated blood pressure (Sys/diaBP of PHAII mice was 125.1 2.5 / 92 2.5 mmHg (n=6) and of WT mice was 117.4 0.6 / 85.5 1.4 mmHg (n=14), Psys<0.0005, Pdia<0.005), higher serum K+, Cl- and Mg+, lower bicarbonate levels, lower renin, and hypercalciuria. The levels of sodium and calcium were normal. In cotrast, the control animals carrying two copies of the WT transgene (WTpol) had lower BP and higher serum Cl- and all other parameters were normal. qPCR and immunohistochemistry of mouse kidney sections stained with anti-NCCT and anti-Calbindin D-28K antibodies (a marker of the distal nephron) revealed increased expression of NCCT in PHAII mice. These features recapitulate PHAII in humans and comfirm that the mutation is a gain-of-function. These data indicate the essential role of WNK4 in determining the balance between Na+ reabsorption and K+ secretion in the distal nephron.

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Epistasic Effects of the Gene Encoding the Gamma Subunit of cGMP-phosphodiesterase on Retinal Dystrophies.

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The gamma subunit of rod phosphodiesterase (PDE6gamma) inhibits the catalytic alpha and beta subunits in darkness; binding of activated GNAT1-GTP removes this inhibition, stimulating PDE activity. PDE6gamma has also been shown to act as a GTPase Accelerating Protein (GAP) for GNAT1-GTP by potentiating the action of another GAP, Regulator of G-protein Signaling 9 (RGS9). Previous experiments have suggested that excess PDE6gamma could turn off transduction and modulate the decay of the light response. We have tested this idea by over-expressing wild-type PDE6gamma in a transgenic mouse. While Pde6gtm1/Pde6gtm1mice mice have severe photoreceptor degeneration as in human retinitis pigmentosa, over-expression of the wild type transgene in the knockout Pde6gtm1/Pde6gtm1mice background prevents this degeneration. Rods with overexpressed PDE6gamma were desensitized ~4-fold compared to wild-type controls; the intensity of light (20ms flashes, 500 nm) needed for 50 percent of saturation was about 6 photons/microm2 for controls, but about 25 photons/microm2 for PDE6gamma over-expressors. Single photon analysis revealed that the light response wave form of PDE6gamma over-expressors was similar to wild-type during the rising phase of the response, but recovery of current following the flash occurred more rapidly. The single photon time-to-peak for controls was about 200 ms, with a full return of the current to the dark level at about 1 s; whereas the PDE6gamma over expressers had a time-to-peak of 150 ms, and a return of current to the dark level at about 0.5s. Acceleration of the turn-off of PDE then speeds the waveform of decay of the light response. Humans or mice with defects in the RGS9 also deactivates of their phototransduction cascades slowly. Patients with RGS9 defects generally complain of being completely blinded by rapid changes in light levels, as when they drive into a tunnel. This bradyopsia phenotype could be so severe that it necessitated immobility or assistance for a few seconds. However, introduction of our PDE6gamma allele masks this bradyopsia phenotype.

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Dynamic Expression of Hsp27 in the presence of mutant ataxin-3. *M. Hsieh[1, 2], W.H. Chang[1], C. Cemal[3], Y.H. Hsu[4], N. Nukina[5], C.S. Liu[6].* 1) Dept Life Sci, Tunghai University, Taichung; 2) Life Science Research Center, Tunghai University, Taichung, Taiwan; 3) Hereditary Ataxia Research Group, Imperial College of Science, Technology and Medicine London SW7 2AZ, UK; 4) Department of Medicine, Tzu-Chi University, Hualien, Taiwan; 5) Laboratory for Structural Neuropathology, RIKEN Brain Science Institute, RIKEN, Wako-shi, Japan; 6) Department of Neurology, Changhua Christian Hospital, Changhua, Taiwan.

Machado-Joseph disease (MJD)/Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominant spinocerebellar degeneration characterized by a wide range of clinical manifestations. In this study, human SK-N-SH neuroblastoma cells stably transfected with full-length MJD with 78 CAG repeats were assayed for the dynamic expression of Hsp27, known as a suppressor of poly (Q) mediated cell death, in the presence of mutant ataxin-3 in different passages of cultured cells. A dramatic decrease of Hsp27 expression was observed in the earlier passage of cultured SK-N-SH-MJD78 cells, however, the later passage of cells showed a significant increase of Hsp27 to almost the same level of the parental cells. Furthermore, immunohistochemical analysis of MJD transgenic mice and post-mortem human brain tissues showed increased expression of Hsp27 compared to normal control brain, suggesting an up-regulation of Hsp27 in the end stage of MJD. However, mutant cells of earlier passages were more susceptible to serum deprivation than mutant cells of later passages, indicating weak tolerance toward stress in cells with reduced Hsp27. Taken together, we proposed that during the early disease stage, the reduction of Hsp27 synthesis mitigated the ability of neuron cells to cope with cytotoxicity induced by mutant ataxin-3, triggering the cell death process during the disease progress. In the late stage of disease, after prolonged stressful conditions of polyglutamine cytotoxicity, the increased level of Hsp27 may reflect a dynamic process of the survived cells to unfold and remove mutant ataxin-3. However, this increased Hsp27 still cannot reverse the global dysfunction of cellular proteins due to accumulation of cytotoxic effects.

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Somatic instability of expanded triplet repeats occurs after organogenesis and is tissue and age-dependent. *I. De Biase[1], R.M. Clark[1], S. Al-Mahdawi[2], A. Monticelli[3], S. Cocozza[3], M. Pook[2], S.I. Bidichandani[1].* 1) Dept Biochemistry & Molecular Biology, Univ Oklahoma HSC, Oklahoma City, OK; 2) Dept of Medical Genetics, Imperial College, London, UK; 3) Dept of Molecular and Cellular Biology and Pathology, Frederico II University, Naples, Italy.

Friedreich ataxia (FRDA) is characterized by gait and limb ataxia associated with cardiomyopathy and diabetes mellitus. The primary sites of pathology are the dorsal root ganglia (DRG). Loss of Purkinje cells in the cerebellum has been described late in disease. FRDA is caused by an abnormal expansion of a GAA triplet repeat sequence (GAA-TR). Repeat length is the main determinant of phenotypic severity. When expanded, the GAA-TR is highly unstable in somatic and germ cells. Using small pool PCR (SP-PCR) analysis, which allows us to detect changes in repeat length in individual chromosomes, we examined whether there are tissue-specific differences in GAA-TR instability. We analyzed multiple tissues from three autopsies of FRDA patients. As previously described in leukocytes, all the tissues analyzed showed a contraction bias. Instability did not correlate with proliferative capacity. However, the frequency and magnitude of expansions was higher in DRG and in cerebellum. To confirm our data, we used a transgenic mouse model, carrying expanded GAA-TR tract within the context of the human FRDA gene. Interestingly, the only tissue that showed significant instability was the cerebellum, although mouse DRG samples were not available for analysis. Our data indicate that non-replication dependent mechanisms have a role in mediating triplet-repeat instability. A higher level of instability was seen in two-month old mice compared to that observed in twelve-month old mice, indicating that instability is also age-dependent. To further address this issue, SP-PCR analysis was performed in multiple tissues from an 18-week fetus with a molecular diagnosis of FRDA, which revealed very low levels of somatic instability compared to corresponding adult tissues. Taken together, our data indicate that somatic instability occurs after organogenesis. Expansions of high magnitude develop in DRG and cerebellum and the process is age-dependent.

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Molecular genetic analysis of Spanish Spinocerebellar Ataxias (SCA). *V. Volpini[1], H.S. Nicolás[1], J. Corral[1], I. Banchs[1], O. Combarros[2], J. Berciano[2], D. Genís[3].* 1) Molecular Genetic Diagnosis , Cancer Research Inst - IDIBELL, Barcelona, Spain; 2) Neurology Service. Valdecillas Hospital. Santander, Spain; 3) Neurology Unit. Josep Trueta Hospital. Girona, Spain.

Autosomal dominant cerebellar ataxias (ADCA) are a clinically heterogeneous group of neurodegenerative disorders caused by unstable trinucleotide repeat expansions. Sevent spinocerebellar ataxia genes: SCAs1-3, SCAs6-7, SCA12 and SCA17 have been cloned with the finding of an expansion of a CAG repeat which encodes a polyglutamine tract. The exception is SCA8, which consists of an exonic but untranslated CTG repeat. We present here the molecular analysis of 237 unrelated familial and 576 sporadic and idiopathic Spanish cases of spinocerebellar ataxia. Over the ADCA familial cases in which we have found a triplet expansion mutation, 7,93% were SCA1; 30,69% SCA2; 34,65% SCA3; 4,95% SCA6; 8,91% SCA7; 11,88% SCA8; and 0,99% SCA17. We have found 12 familial index cases with SCA8 expansions whose expansion allele range goes from 86 to 184 repeats (110,97 22,13; Pearson Coef. =19,93%). Maternal transmissions presented elongations of the triplet CTG sequence ranging from +2 to +13 repeats (7,5 5,5; Pearson Coef = 73,33%). In contrast, paternal transmissions presented contractions ranging from -1 to -17 repeats (-9,75 5,97; Pearson Coef = -61,23%). We have found several giant SCA8 expansions in our casuistic, and all of them were present in unaffected adult individuals. The giant SCA8 expansions seems to be originated from homozygous SCA8 females with alleles of moderate expanded size; whereas homozygous males in the same allele range size have transmited contracted alleles to their respective offspring, as in heterozygous cases occurs. None of SCA mutated cases also coexists with SCA8 expansions in their reported pathogenetic range. About 60% of familial ADCA cases remained genetically unclassified. No SCA mutations were detected in the 576 isolated and idiopathic cases of spinocerebellar ataxia.

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Expanded FMR1 CGG repeats induce intranuclear inclusion formation and abnormal lamin A/C organization in human cultured cells. *D. Garcia Arocena[1,2], C. Iwahashi, S. Beilina[1], N. Won[1], F. Tassone[1], P.J. Hagerman[1].* 1) Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, CA; 2) Departamento de Genetica, Facultad de Medicina, UDELAR, Montevideo, Uruguay.

Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder affecting some adult carriers of premutation alleles (55-200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene. FXTAS is thought to be caused by a toxic gain of function of the expanded-CGG-repeat *FMR1* mRNA, which is found in the neuronal and astrocytic intranuclear inclusions that are associated with the disorder. Using *FMR1* 5untranslated region (5UTR) reporter constructs with CGG repeats in the premutation range, we have demonstrated that intranuclear inclusions can be formed in both primary astrocytic and long-term established neural cell cultures. As with the inclusions found in post-mortem tissue, the inclusions induced by the expanded CGG repeat are B-crystallin-positive; however, inclusions in culture are not associated with ubiquitin, suggesting that incorporation of ubiquitinated proteins may be a later event in the disease process. The absence of ubiquitinated proteins suggests further that inclusion formation does not reflect a failure of the proteasomal degradative machinery. The presence of the expanded CGG repeat, as RNA, results in reduced cell viability as well as the disruption of the normal architecture of lamin A/C within the nucleus. This last observation, and the findings that lamin A/C is present in both the inclusions of FXTAS patients and the inclusions in cell culture, suggests that lamin A/C dysregulation may be a mediator of FXTAS. This work was funded by the National Institute of Child Health and Development (HD40661, PJH).

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Molecular consequences of PHOX2B missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. *D. Trochet[1], S.J Hong[2], J.K LIM[2], J.F Brunet[3], A. Munnich[1], K.S Kim[2], S. Lyonnet[1], C. Goridis[3], J. Amiel[1].* 1) Genetics, INSERM U393, Hosp Necker, Paris, France; 2) Molecular Neurobiology, MRC 216, Harvard Medical School, USA; 3) CNRS, UMR 8542, ENS, Paris, France.

Heterozygous mutations of the PHOX2B gene account for a broad variety of disorders of the autonomic nervous system (ANS), either isolated or combined, including congenital central hypoventilation syndrome (CCHS), tumours of the sympathetic nervous system (TSNS) and Hirschsprung disease. In CCHS, the prevalent mutation is an expansion of the 20 alanine stretch ranging from +5 to +13 alanines, while frameshift and missense mutations are found occasionally. To determine the molecular basis for impaired PHOX2B function, we assayed the transactivation and DNA binding properties of wild-type and mutant PHOX2B proteins. Furthermore, we investigated aggregate formation by proteins with a polyalanine tract expansion ranging from +5 to +13 alanines using immunofluorescence of transfected cells and gel filtration of in vitro translated proteins. We found that transactivation of the dopamine beta-hydroxylase promoter, a well characterized natural PHOX2B target, by PHOX2B proteins with frameshift and missense mutations is abolished or severely curtailed, as is in vitro DNA binding. However, the missense and frameshift mutations tested showed a nuclear localization, excluding protein instabity. The transactivation potential of proteins with polyalanine tract expansions declines with increasing length of the polyalanine stretch, starting with the +6 alanines allele, while in vitro DNA binding was affected for an expansion of +9 alanines and above. Cytoplasmic aggregation in transfected cells is observed only for the longest expansions, although the +5 alanines mutant is already more prone to form multimers in vitro than the wild-type protein. Such a tendency to protein misfolding (leading at most to nuclear exclusion) could explain loss of transactivation for alanine expansion mutations. Altogether, our data show a loss of transactivation irrespective of the nature of the mutation, although the disease-causing mechanism seems to vary according to the nature of the mutation.

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An enhanced PCR assay to detect pre- and full mutation alleles of the *FMR1* gene. *A. Saluto[1], A. Brussino[1], F. Tassone[2], C. Arduino[3], C. Cagnoli[1], P. Pappi[3], P.J. Hagerman[2], N. Migone[1,3], A. Brusco[1,3].* 1) Dept. Genetics Biology & Biochemistry, University of Turin, Turin, Italy; 2) Department of Biochemistry and Molecular Medicine, University of California, Davis, California 95616, USA; 3) Medical Genetics Unit, San Giovanni Battista Hospital, Turin.

Several diagnostic strategies have been applied to the detection of *FMR1* gene repeat expansions. Here we report a novel PCR strategy using the Expand Long Template PCR kit (Roche Diagnostics) and betaine. Repeat expansions up to 330 CGGs in males and to at least 160 CGGs in carrier women could be easily visualized on ethidium bromide agarose after amplification with standard primers c and f (Fu et al., 1991), the buffer 2 (2.25 mM $MgCl_2$) and 1.7 M betaine. Fluorescence analysis of PCR products in both male and females with pre- (26 subjects, range 55-200 CGG) and full mutations (13 subjects, 200 CGG) showed a peculiar profile with an array of stutter bands, which were never observed in over 178 controls (11-54 CGG). These series of peaks therefore appear to be specific for the presence of expanded alleles, although the lower limit of this phenomenon has not been defined. We sequenced the *FMR1* repeat in 18 subjects and 10 clones containing the *FMR1* repeated region, and demonstrated that there is a direct, approximately linear correlation between the size of the PCR fragments - measured by capillary electrophoresis - and the number of repeats, with a 212.4 bp apparent size of the constant region of instead of the real 221 bp, and an increase of 2.7 bp for each additional triplet. We provide a quick reference ladder to estimate the number of CGG repeats from the apparent size of the PCR fragment up to 100 triplets. Furthermore, we noted that an intrinsic measurement error (1 triplet), probably due to technical artifacts, is always present. Southern blot methods are still necessary to size the number of expanded repeats above 100 CGG, and to evaluate the methylation status of the expanded allele; however, the use of this fluorescence method will dramatically reduce the number of Southerns required for screening studies. Grants acknowledgment: Regione Piemonte CIPE36/2002 N.Migone, N.I.H. HD40661 P.J.Hagerman.

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Screening of a Compound Library to Identify Modifiers of Ataxin-1 Phosphorylation. *K.M. Carlson[1], M.D. Kaytor[1], O. Rainwater[1], C.E. Byam[1], H.Y. Zoghbi[2], H.T. Orr[1].* 1) Lab Medicine and Pathology, Institute of Human Genetics, Univ of Minnesota, Minneapolis, MN; 2) Howard Hughes Medical Institute, Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Spinocerebellar Ataxia-Type 1 (SCA1) is an autosomal dominant neurodegenerative disease resulting from a glutamine tract expansion in the ataxin-1 protein. Studies implicate both inherent properties of the mutant ataxin-1 protein as well as misregulation of cellular pathways in SCA1 pathogenesis. S776 of ataxin-1 is phosphorylated and has been demonstrated to be an important intragenic modifier of SCA1 pathogenesis in vivo. Previously, a cell-based assay was developed to identify compounds that alter ataxin-1 phosphorylation. In this assay, stably transfected cell lines expressing GFP-tagged ataxin-1 are exposed to a compound of interest. GFP levels are measured to determine the total amount of ataxin-1. An ELISA, using an antibody specific to ataxin-1-p776, is performed to measure the amount of pS776-ataxin1. Using this assay, we now report the results of a screen against a library of 2000 compounds (The Spectrum Collection) made up of natural and FDA approved agents. A screen at 10uM was performed in triplicate against both mutant and wild type ataxin-1. 333 compounds were identified that altered the phosphorylation of mutant ataxin-1 specifically. Of these compounds, 35 decreased phosphorylation by greater than 50%. We identified 88 compounds that altered the phosphorylation state of both mutant and wild type ataxin-1. Finally, we identified 83 compounds that altered the phosphorylation state of wild type ataxin-1 only. Classes of compounds identified in our screen include anti-inflammatory, antineoplastic agents, and antibiotics. We are currently working to prioritize our hits for further studies. The identification of compounds that modulate ataxin-1 S776 phosphorylation will provide us with a greater understanding of the signaling pathways involved in both the wild type function of ataxin-1 as well as SCA1 pathogenesis. In addition, compounds identified in this screen might be good candidates for future studies aimed at identifying effective therapeutics for SCA1.

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The impact of polyglutamine expansion on TBP function. *M.J. Friedman[1,2], SH. Li[2], XJ. Li[2].* 1) Graduate Program in Genetics and Molecular Biology; 2) Dept. Human Genetics, Emory University, Atlanta, GA., USA.

Transcriptional dysregulation is widely thought to be involved in the molecular pathogenesis of the polyglutamine (polyQ) diseases. In most of the polyQ diseases, the mutant protein or a polyQ-containing fragment thereof can localize to the nucleus. Study of spinocerebellar ataxia 17 (SCA17), which is caused by a polyglutamine expansion in the TATA-binding protein (TBP), may provide insight into this pathogenic mechanism. TBP mediates transcription by all three eukaryotic RNA polymerases as a fundamental component of multiple nuclear complexes. Unlike the C-terminal domain (CTD) of TBP, the function of the evolutionarily divergent N-terminus as well as the polyglutamine tract therein is largely unknown. To gain insight into the functional importance of this polyQ tract, we have generated TBP cDNA constructs containing 15, 31, 71, and 105 CAGs. When expressed in HEK 293 cells, only mutant TBP (71 and 105 CAGs) formed nuclear aggregates. Reporter assays revealed an elevated transactivation capacity for polyQ-expanded TBP at TATA-containing promoters. The same effect was not observed at a RNA polymerase II promoter that lacks a TATA box. In addition, polyQ expansion antagonized the formation of transcription-incompetent TBP dimers. These results are consistent with a gain-of-function phenomenon in which polyQ expansion, presumably by inducing a conformational change, unmasks the activation potential of the N-terminus of TBP and interferes with normal transcriptional regulation.

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Decreased palmitoylation of mutant huntingtin leads to disturbed trafficking and increased neuronal toxicity *in vivo*. *A. Yanai[1], R. Kang[2], K. Huang[2], R.R. Singaraja[1], P. Arstikaitis[2], L. Gan[1], P.C. Orban[1], A. Mullard[2], R.C. Drisdel[3], W.N. Green[3], A. El-Husseini[2], M.R. Hayden[1].* 1) Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada; 2) Department of Psychiatry, University of British Columbia, Vancouver, Canada; 3) Department of Neurobiology, Pharmacology and Physiology, University of Chicago, Chicago, IL.

Expansion of the polyglutamine tract of huntingtin (htt) is the mutation underlying Huntington Disease (HD). Htt is associated with specific intracellular membranes and interacts with proteins involved in vesicle trafficking. Post-translational modification by the fatty acid palmitate is an important and reversible modification, crucial for correct sorting and functional modulation of different membrane proteins and their signaling pathways. The association of htt with detergent resistant membranes (rafts) as well as its interaction with the palmitoyl transferase HIP14 raised the possibility that palmitoylation of htt may be an important mechanism for regulating its trafficking and function.

Here we show that htt is palmitoylated at cysteine 214. Furthermore, palmitoylation is modulated by CAG size as demonstrated by a marked decrease in palmitoylation of mutant htt from HD mouse brain ($p=0.01$). Trafficking of palmitoylation resistant wt and mutant htt is disturbed in COS and HEK293 cells as well as in primary neuronal cultures. Reduced palmitoylation of mutant htt in an HD mouse model and down regulation of HIP14 in neurons from HD mouse brain are associated with disturbed trafficking of mutant htt, leading to a significant increase ($p=0.002$) in inclusion body formation. Moreover, palmitoylation-resistant wt and expanded htt show a marked increase (23%; $p=0.01$ and 28%; $p=0.03$, respectively) in neuronal toxicity. Decreased palmitoylation of mutant htt and its effect on trafficking and neuronal toxicity identifies a novel pathway in the pathogenesis of HD.

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Large untranslatable CAG repeats are pathogenic in vivo: using Caenorhabditis elegans as a model system. *K.-M. Hsiao[1], K.-Y. Chen[2], H. Pan[1], L.-C. Wang[1].* 1) Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan.

Recent studies on the molecular mechanisms of myotonic dystrophy (DM) as well as on the secondary structure formed by expanded CAG repeats and its toxic effects raise the possibility that RNA repeats, in addition to CUG repeats, may play a prominent role in trinucleotide repeat diseases. Here, we used the nematode *Caenorhabditis elegans* as a model system to test this hypothesis by expression of green fluorescent protein gene (*GFP*) with various lengths (5, 30, 83, 120, and 200) of untranslated CAG and CTG repeats under the control of body wall muscle-specific promoter. Interestingly, both CAG and CTG repeats cause size-dependent pathogenic effects with a threshold of more than 83 repeats. Animals expressing GFP transcripts with 120 repeats, although developed normally, showed age-dependent abnormality of muscle structure, uncoordinated motility, shortened life span, and much lower brood size than those expressing shorter repeats. Animals expressing 200 or more repeats either died during embryogenesis or showed retarded growth at early larval stages, and thus could not produce offspring. Although the GFP expression was reduced in animals expressing large repeats, the RNA levels remained unaltered. These results strongly suggest that in addition to the CTG repeats, the expansion of untranslated CAG repeats is deleterious to living organisms as well. Furthermore, they show that pathogenic threshold of untranslated CAG repeats seems longer than those frequently observed in polyglutamine disease genes, indicating that RNA pathogenesis may not play a major role in those diseases.

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Long tract of untranslated CAG repeat is pathogenic in transgenic mice. *H. Pan[1], R.-J. Hsu[2], C.-Y. Li[2], K.-M. Hsiao[1].* 1) Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan.

The most frequent trinucleotide repeats found in human disorders are CAG and its complementary sequence, CTG. CAG repeats are mostly found in coding regions whereas CTG expansions are located in untranslated regions. To investigate whether an untranslated CAG repeat expansion has pathogenic effect *in vivo*, we generated transgenic mice expressing muscle-specific *EGFP* transcripts with different sizes of CAG repeats in its 3-untranslated region. While the expression levels of the *EGFP* transcripts were comparable in all transgenic lines, the EGFP protein levels were significantly reduced in mice expressing 200 CAG repeats. Histological analysis of the muscle revealed atypical cell morphology, as well as altered activities of succinate dehydrogenase and NADH reductase in CAG_{200} mice. These mice showed signs of low grip strength, less cage activity, and reduced fertility. The sperm counts of male CAG_{200} mice were normal but the sperm motility was significantly decreased. The reduction of sperm motility was associated with structural and mitochondrial defects in the sperm tails. Furthermore, abnormal muscle contracture was elicited in the skeletal muscle of CAG_{200} mice, suggesting an impaired energy metabolism. These results demonstrate, for the first time, that untranslated CAG repeat expansion in an unrelated mRNA can have pathogenic effects *in vivo*, consistent with the RNA gain-of-function model. It also suggests a possible role for untranslated CAG expansion in human disorders.

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A novel missense mutation in the Sodium Bicarbonate Cotransporter (NBCe1/SLC4A4) in a patient with proximal renal tubular acidosis and ocular pathology. *M.B. Gorin[1, 2], F.Y. Demirci[1], M-H. Chang[3], T.S. Mah[1], M.F. Romero[3, 4].* 1) Dept. of Ophthalmology, UPMC Eye Center, Ophthalmology and Visual Science Research Center, Univ. of Pittsburgh SOM, Pittsburgh, PA; 2) Dept. of Human Genetics, Univ. of Pittsburgh GSPH, Pittsburgh, PA; 3) Dept. of Physiology and Biophysics, Case Western Reserve Univ., Cleveland, OH; 4) Dept. of Pharmacology, Case Western Reserve Univ., Cleveland, OH.

The electrogenic Na^+/HCO_3^- cotransporter (NBCe1/SLC4A4) plays a major role in renal bicarbonate absorption via the proximal tubules that is crucial for maintaining the normal blood pH. The human NBCe1 (chr 4q21) produces 2 major variants by alternative promoter usage [kNBCe1 (cryptic promoter) is originally cloned from kidney, pNBCe1 (normal promoter) is pancreatic/general form]. Though rare, recessive NBCe1 mutations have been reported for a small number of patients with proximal renal tubular acidosis (pRTA), short stature, and ocular pathology. A 27 year-old adopted male patient with no available family history presented with pRTA, short stature, and bilateral ocular disease (cataract, glaucoma, and band keratopathy). Using direct PCR sequencing, we screened the NBCe1 exons and identified a novel, homozygous, missense mutation (Leu522Pro in kNBCe1). We functionally characterized this mutation by expressing the wild-type NBCe1 and the L522P-NBCe1 in Xenopus oocytes. When injected into oocytes, the mutant RNA failed to induce electrogenic transport activity. Since L522 is located in the middle of transmembrane span 4, the L522P-protein is predicted to not properly fold for membrane insertion. Further experimentation showed that the L522P-protein is not effectively transported to the oocyte membrane and thus is unable to act as a transmembrane transporter. These data would explain both the insufficient renal bicarbonate absorption (proximal RTA) and the inappropriate ocular fluid transport (cataract, glaucoma, band keratopathy) in this patient. Identification and characterization of novel mutations increase our understanding of the structural-functional aspects of the NBCe1 and the molecular basis of the multi-organ pathologies associated with NBCe1 defects.

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Linkage studies in an Ecuadorian population with Keratoconus. *R.A. Lewis[1], M. Gajecka[2], D. Winters[2], A. Molinari[3], J.A. Pitarque[3], M.H. Chahrour[1], S.M. Leal[1], B.A. Bejjani[2,4].* 1) Cullen Eye Institute, Baylor College of Medicine, Houston, TX; 2) Health Research and Education Center, Washington State University, Spokane; 3) Hospital Metropolitano, Quito, Ecuador; 4) Sacred Heart Medical Center, Spokane, WA.

Keratoconus (KC) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers, and altered visual acuity. Although both genetic and non-genetic factors have been associated with KC, its molecular basis is still elusive. We identified an Ecuadorian cohort in which KC without other ocular or systemic features is transmitted as an autosomal dominant trait with incomplete penetrance. Here we present the results of sequencing and linkage analyses which were performed to verify the association between KC in these families and one or more of the previously reported KC loci. To date, we have examined, collected blood, and purified DNA from 68 affected individuals, 75 unaffected individuals and 27 unknown/young/possible individuals from 23 multiplex families with KC. Subjects were diagnosed clinically with KC by slit lamp examination and corneal topography. We excluded previously assigned KC loci on chromosomes 2, 3, 15, 16, and 20 by linkage analysis and finished a genome-wide screen with an average spacing of 10 cM for a KC locus. We found no evidence for linkage between KC and the markers tested. Recently, a second genome-wide screen with a more dense marker set was performed. We have genotyped 170 individuals with fluorescent markers with an average spacing of 5 cM spanning chromosomes 3, 4 and 15-22. Data are being generated before linkage analysis. Additionally, the coding exons of *VSX1* in 60 affected individuals from the Ecuadorian families and two ethnically matched control individuals (Ecuadorian individuals with no ocular abnormalities) were sequenced. Three single nucleotide polymorphisms (SNPs) in the *VSX1* coding region (18GT, 174GT and 542AG) were identified, but no mutation was found in the gene. Keratoconus in our families is not linked to any of the previously defined KC loci. A genome-wide screen is in progress.

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Identifying familial high myopia candidate genes in a Polish population. *A. Frajdenberg[1], M. Rydzanicz[2], M. Podfigurna-Musielak[3], S.M. Leal[4], B.A. Bejjani[5,6], K. Pecold[1], M. Gajecka[2,5].* 1) Department of Ophthalmology, Marcinkowski University of Medical Sciences, Poznan, Poland; 2) Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; 3) Department of Ophthalmology, Lezno Hospital, Lezno, Poland; 4) Baylor College of Medicine, Houston, TX; 5) Health Research and Education Center, Washington State University, Spokane; 6) Sacred Heart Medical Center, Spokane, WA.

Myopia is the most common of all ocular conditions. Affecting about one-fourth of the world population, it constitutes a significant public health problem. Although high myopia (myopia in excess of 6.0 diopters [D]) is far more rare than mild/moderate myopia, the importance of high myopia is significant because its development involves anterior-posterior enlargement of the eye, abnormal changes in the eye and frequent detachment of the retina. The etiology of myopia is not known, but both genetic and environmental factors seem to play a role. In high myopia, genetic factors appear to play a predominant role. Nineteen Polish families have been ascertained which appear to have myopia that is inherited as an autosomal dominant trait. To date, we have examined, collected blood, and purified DNA from 110 individuals from these families, each with multiple individuals with high myopia. Simulation studies revealed that the families have sufficient power to establish linkage in these families which may lead to the identification of familial high myopia gene(s). Preliminary genotyping of high myopia associated loci [7q36 (*MYP4*), 12q21-23 (*MYP3*), 18p11.31 (*MYP2*), and 17q21-23 (*MYP5*)] in the Polish families by genotyping of all subjects with well-spaced polymorphic markers was initiated. Additionally, evaluation of the candidate genes for familial high myopia by sequence analysis of *LUM*, *FMOD* and *TGIF* genes in the Polish families was performed.

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Prevalence of retinal dehydrogenase 12 (RDH12) mutations in Leber congenital amaurosis and genotype-phenotype correlations. *I. Perrault[1], S. Hanein[1], S. Gerber[1], H. Dollfus[2], C. Hamel[3], J.L. Dufier[4], A. Munnich[1], J. Kaplan[1], J.M. Rozet[1].* 1) Dept Genetics, INSERM U393, Hopital Necker, Paris, France; 2) Dept Genetics, Hopital Hautepierre, Strasbourg, France; 3) Neurosciences, INSERM U583, Montpellier France; 4) Ophthalmology, Hopital Necker, Paris, France.

Leber congenital amaurosis (LCA), the most early-onset and severe form of all inherited retinal dystrophies, is responsible for congenital blindness. Eleven LCA genes have been mapped, and eight of these have been identified. Recently, we reported the respective frequencies of mutations in each of the first seven LCA genes in a series of 179 unrelated patients. The purpose of this study was to determine the prevalence of mutations in the newly identified LCA gene, RDH12, in an enlarged series of 300 patients affected with LCA. The 7 exons encoding RDH12 were screened for mutations by DHPLC and direct sequencing using specific primers designed from intronic, 5 and 3 UTR sequences. Patients to be screened at first were selected on the basis of their retinal phenotype i.e. affected with the "severe yet progressive rod dystrophy" form of the disease (LCA subtype II). Mutations were identified in 13/300 patients of our series (4.3 %). When patients affected with the second subtype of the disease were considered alone, mutations of RDH12 accounted for 10.8% (13/119) of cases. This study demonstrates that the natural history of the disease since birth, associated with the light behaviour of patients, the refraction data and the description of the fundus are essential to selected LCA genes to screen in priority and thus to lighten the molecular diagnosis of this highly genetically heterogeneous condition.

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Striking increased expression of endothelin-2 in mouse models of inherited photoreceptor degenerations (IPDs).

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Although more than 150 genes have been shown to lead to IPD, the mechanisms underlying the apoptotic death of the photoreceptors (PRs) in these disorders is unknown. We showed previously that the mutant PRs are at a constant increased risk of death, but can nevertheless function normally for years to decades in humans (Pacione et al. Ann Rev Ns 2003). To account for these findings, we proposed the mutant steady-state (MSS) hypothesis (Clarke et al., 2000), suggesting that changes in the abundance or activity of one or more mutant response proteins may confer or resist the increased risk of death. To identify differentially expressed genes in IPDs, we performed microarray and real-time PCR experiments. We established that the gene for endothelin-2 (*Edn2*) was up-regulated 32-fold in the $Rds^{+/-}$ mouse model and 14-fold in a *RHO P347S* mouse model of IPD. Endothelin-2 belongs to the family of endothelin neuropeptides (i.e. endothelin-1, endothelin-2, endothelin-3), and is a potent vasoconstrictor. Whereas *wt* retinas gave virtually no in situ hybridization signal for *Edn2* transcripts, a strong *Edn2* signal was found in the PRs, but no other retinal cell types, of $Rds^{+/-}$ mice. Moreover, we found that the concentration of total endothelin peptides, determined by radioimmunoassay (RIA) was 3.2-fold increased in the retinas of $Rds^{+/-}$ mice: 0.122 fmol/retina for *wt* retinas vs. 0.395 fmol/retina for $Rds^{+/-}$ retinas (12 retinas per genotype). Since neither *Edn1* nor *Edn3* mRNA was increased by real-time PCR in $Rds^{+/-}$ retinas, the increase in total endothelin in $Rds^{+/-}$ retinas is most likely due an increase in Edn2 peptide alone. We conclude that i) the mutant PRs in IPDs have a dramatically increased expression of *Edn2* mRNA, ii) this increase is translated into increases in the abundance of the Edn2 peptide, and iii) the expression of Edn2 in stressed PR neurons is likely to be a survival response that resists the death of these cells.

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Development of Dominant Cone Dystrophy Depends on Expression Levels of GCAP1 (Y99C) in Transgenic Mice. *N. Roychowdhury[1], S. Thirumalaichary[1], K. Zhang[2], J. Frederick[1], W. Baehr[1].* 1) Dept Ophthalmology, Moran Eye Ctr, Salt Lake City, UT; 2) University of California San Francisco, San Francisco.

Photoreceptor Guanylate Cyclase Activating Protein 1 (GCAP1) is a Ca2+ -binding protein belonging to the calmodulin gene family with four EF hand motifs (EF1-4). Several mutations in EF3 and EF4 of GCAP1 have been linked to autosomal dominant cone dystrophy and cone-rod dystrophy in independent pedigrees. We generated mice expressing a transgene consisting of the entire GCAP1 (Y99C) gene including upstream and downstream regions, and EGFP fused to the C-terminal of mutant GCAP1. Different lines with various levels of expression of Y99C-GFP were obtained. Lines with high levels of mutant GCAP1 expression showed expression of green fluorescent mutant GCAP1 in rods and cones, while a low expressor line expressed exclusively in cone photoreceptors. High expressor lines exhibited a slow cone-rod dystrophy developing over a time range of 15 months, as judged by electroretinography and histology. The low expressing line (approximately 10% of normal levels) showed no cone dystrophy even at age one year. This line was used for cone mapping studies in whole retina mounts. The distribution pattern of cones in the retina was shown to be uneven, with green fluorescent cones more concentrated towards inferior-nasal part. The results show that the development of a dominant cone dystrophy depends on mutant GCAP1 expression levels.

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ARHGEF9: An Xcellent mental retardation candidate gene. *E.J. Marco[1], J. Bristow[2], P.D. Cotter[3], R.E. Stevenson[4], L. Pennacchio[2], C.E. Schwartz[4], E.H. Sherr[1].* 1) Dept Neurology, Univ California, San Francisco, San Francisco, CA; 2) Joint Genome Institute, LBNL, Berkeley, CA; 3) Dept of Medical Genetics, Univ California, San Francisco, CA; 4) Geenwood Genetic Center, Greenwood, SC.

Our study aimed to identify the genes affected by a chromosomal rearrangement in a patient with mental retardation. We evaluated a non-dysmorphic female with mental retardation (FSIQ 54) and a paracentric X chromosome inversion (46,X, inv (X) (q13.1; q26.3). The inverted X underwent early replication in 100/100 lymphocytes studied. Metabolic testing and a high resolution MRI were unrevealing. Using fluorescent in situ hybridization with bacterial artificial chromosomes, we found that the centromeric breakpoint transected ARHGEF9, a RhoGEF encoding gene. The telomeric breakpoint resided in a gene poor region. Based on this finding, we sequenced the ARHGEF9 coding region and intron/exon boundaries in 384 individuals (99 probands from families with X-linked mental retardation and 285 males with non-Fragile X mental retardation.) We found a splice site change (IVS5+5 g>a) in a male from the unlinked group. This G in the 5'splice site is quite invariant. Similar mutations are known to cause alternate splicing in other disease associated genes (i.e. SMS and SCL6A8.) We also identified a non-synonymous polymorphism (I10V) in brothers from an X-linked family. Isoleucine in this position is highly conserved among vertebrates and occurs at the start of the SH3 domain. Neither of these changes has been reported in the dbSNP database (build 124.) Investigation is underway to assess the functional significance of these changes and further characterize the phenotype of affected individuals.

ARHGEF9 (Xq11.1) encodes Collybistin (hPEM) which activates the Rho GTPase, Cdc42. It is ubiquitously expressed in the human brain and may regulate actin cytoskeleton dynamics and cell signaling. A mutation in this gene has been previously associated with hyperekplexia and epilepsy. A related gene, ARHGEF6, has already been linked to non-syndromic mental retardation. This knowledge plus our results strongly suggest a role for ARHGEF9 in non-syndromic X-linked mental retardation.

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Identification of differentially expressed genes in RNA inhibition by siRNA against *MECP2*. *I.J. Kim[1], C.R. Kim[4], M.A. Yoo[5], C.M. Kim[1, 2, 3].* 1) Department Biochemistry, College of Medicine, Pusan National University, Busan; 2) BioMedical Informatics Unit, College of Medicine, Pusan National University, Busan; 3) Medical Research Institute, Pusan National University Hospital, Busan; 4) Department Statistics, College of Natural Science, Pusan National University, Busan; 5) Department Molecular Biology, College of Natural Science, Pusan National University, Busan.

Rett syndrome (RTT) is an X-linked dominant childhood neurodevelopmental disorder. *MECP2* has been reported to be the RTT-causing gene. The MeCP2 participates in transcriptional silencing via DNA methylation. We investigated the profile and function of the downstream genes regulated by MeCP2. To identify the MeCP2-regulated genes, we performed RNA inhibition using siRNA against *MECP2* and microarray experiment. We used the linear amplification of the isolated RNA in order to get enough RNA. Amplified aRNA was labeled and hybridized to human 8K cDNA microarray. *MECP2* expression in HEK293 cells was decreased by 65.8% in the siRNA group compared to the control group using semi-quantitative RT-PCR and Western blot. Among the differentially expressed 279 genes, 188 genes were upregulated greater than 2-fold threshold and 91 genes were downregulated after silencing the *MECP2* in HEK293 cells. We found that neurodegenerative disorder-related, metabolismic disorder-related, and apoptosis-regulated genes were overexpressed in *MECP2*-inhibited HEK293 cells. Down-regulated genes were the ones related with cell proliferation, differentiation, and growth, and muscle functions. Of these upregulated genes, c-jun of apoptosis group had the greatest change. Meanwhile cystathionase (CTH2) of cell proliferation/differentiation group had the greatest change of these downregulated genes. Consequently, the differentially expressed genes may be influenced by MeCP2 inhibition, either directly or indirectly, and will provide valuable information for understanding the mechanisms of MeCP2 in gene expression regulation.

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Maternal Inherited Thrombophilia and Fetal Growth Restriction: A Meta-Analysis. *K. King[1], J. Lau[2], L. Demmer[3].* 1) Molecular and Medical Genetics, Oregon Health & Science Univer, Portland, OR; 2) Clinical Care Research, Tufts-NEMC, Boston, MA; 3) Pediatrics, Tufts-NEMC, Boston, MA.

Objective: The purpose of this study was to conduct a systematic review examining the association between intrauterine growth restriction (IUGR) and common inherited thrombophilias, specifically Factor V Leiden (FVL), Prothrombin G20210A mutation (PTM), and homozygous methylenetetrahydrofolate C677T variant (MTHFR).

Study Design: MEDLINE and manual bibliography searches were used to identify case-control and cohort studies evaluating the association between IUGR and three common inherited thrombophilias.

Results: The meta-analysis included 14 studies investigating FVL, 14 studies investigating PTM, and 11 studies investigating MTHFR. There was a significant association between IUGR and FVL (OR 2.07; CI 1.35-3.16), PTM (OR 2.11; CI 1.42-3.15), and MTHFR (OR 1.41; CI 1.07-1.84). The strongest association was between IUGR and FVL homozygotes (OR 4.55; CI 1.10-18.74). Sensitivity analyses were performed which included analysis of (1)the largest studies, (2)different definitions of IUGR, (3)gestational age at the time of delivery, and (4)studies that excluded patients with maternal hypertension or preeclampsia. When patients with maternal hypertension or preeclampsia were excluded, the associations became non-significant (FVL: OR 1.62; CI 0.84-3.10; PTM: OR 1.26; CI 0.51-3.12; MTHFR: OR 1.07; CI 0.52-2.20).

Conclusion: Although there appears to be an association between maternal inherited thrombophilias and IUGR, it is still not fully established. In particular the potential confounding effect of preeclampsia on fetal growth restriction needs to be resolved before setting forth any clinical recommendations based on these findings. It may be reasonable to consider screening for thrombophilia in the setting of early, severe IUGR or IUGR associated with preeclampsia.

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Prenatal diagnosis of a rare radio-ulnar synostosis and amegakaryocytic thrombocytopenia syndrome by ultrasound. *G.G. Fraley[1], A.A. Thompson[2], M.J. Dinsmoor[1], M.J. Kim[1], L. Liu[1], S.N. MacGregor[1], J.S. Sholl[1], P.C. Weiss[1], I.S. Salafsky[1].* 1) Evanston Northwestern Healthcare, Feinberg School of Medicine, Northwestern University, Evanston, IL; 2) Children's Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Radio-ulnar synostosis (RUS) with congenital amegakaryocytic thrombocytopenia is a rare autosomal dominant syndrome associated with HOXA11 mutations. Neonatal thrombocytopenia has been reported in three cases. This is the first reported case with in utero detection of skeletal defects and bone marrow failure. The family was known to be at risk for this condition based on genetic testing and clinical findings in the father, his brother and a paternal niece. Ultrasound examination at 20w 5d revealed abnormal hand alignment. Another scan at 28w 5d revealed crossing of the proximal radius and ulna consistent with RUS and deviation of the phalanges. All other measurements were normal. The option of percutaneous umbilical blood sampling (PUBS) to assess platelet count prenatally was declined by the parents due to the procedure-related risk and absence of previous reports of fetal thrombocytopenia. The patient presented four days later at 29w 2d with decreased fetal movement and hydrops. A male infant weighing 1500 grams was delivered by emergency Cesarean section with Apgars of 1 and 1. The platelet count was 5 thou/cu mm and the hemoglobin was 11.1 gm/dL. The child died on day 1 of life. Preliminary autopsy findings noted moderate non-immune hydrops secondary to anemia, petechial hemorrhage of multiple internal organs including brain, pleural effusion and confirmed the finding of RUS and deviated phalanges. Mutation analysis of the HOXA11 gene and bone marrow histology are pending. We theorize that low platelet levels in utero predisposed this fetus to a hemorrhagic event and led to anemia and hydrops. This is the first reported case of antenatal thrombocytopenia and fetal crisis with this syndrome. Prenatal diagnosis using ultrasound is possible for this syndrome. In affected cases, the risk for antenatal thrombocytopenia should be discussed and PUBS may be considered with possible platelet transfusion.

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PREnatal VENTriculomegaly Clinical Classification(Part 1). *R.D. Wilson[1,2,3], J. Ilagan[1], R. Finkel[1,3], E. Simon[1,3], L. Bilaniuk[1,3], B. Coleman[2,3], S. Horii[2,3], M.P. Johnson[1,2,3], M. Bebbington[1,2,3], N.S. Adzick[1,3].* 1) Ctr Fetal Diag/Treatment/Wood, Children's Hosp Philadelphia,; 2) Hospital of the University of Pennsylvania; 3) University of Pennsylvania, Philadelphia, PA.

Introduction : Prenatal second trimester ultrasound assessment identifies CNS anomalies. Prenatal classification is required as part of an organized follow-up protocol . PREVENT Part 1 presents a classification based on the imaging evaluation of fetuses with a referring diagnosis of ventriculomegaly / hydrocephalus .Methods : Retrospective review of a clinical database with a referring diagnosis of ventriculomegaly / hydrocephalus (IRB 2005-1-4121) using ultrasound and MRI to group fetuses into clinical imaging categories(part 1)and identify groups for longterm follow-up (part 2). Additional clinical information from history , echocardiography , and chromosomes was used to assist with isolated and multiple anomalies .Results : Initial review of CNS referrals identified 123 fetuses with prenatal diagnosis that included ventriculomegaly . Three clinical categories were used cerebral ventricles < 10mm , 10-15mm , and > 15mm with subcategories of symmetric , asymmetric , +/-imaging impression of bleeding (intra or periventricular).

vent size	no pts	Symm	Asymm	Sym blood	Asym blood
< 10 mm	22	7	10	3	2
10-15 mm	65	31	12	13	9
> 15 mm	36	18	5	5	8

Conclusions : Asymmetric ventricles were more likely to have evidence of bleeding (41% vs 27%)while ventricles >15mm were more likely to have additional CNS anomalies. Longterm follow-up/ morbidity is necessary for appropriate prenatal counseling .

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Rapid determination of the genetic origin by quantitative fluorescent PCR in hydatidiform moles. *O. Samura, N. Miharu, M. Hyodo, N. Fujito, M. Tanigawa, Y. Kudo.* Dept OB/GYN, Hiroshima Univ Sch Medicine, Hiroshima, Hiroshima, Japan.

Hydatidiform mole (HM) is classified into partial(PHM) and complete(CHM) subtypes according to histopathologic and genetic criteria. Traditionally, it is believed that PHM carries a better prognosis and rarely develops metastasis. However, making a distinction between PHM and CHM using histologic criteria alone may be difficult. Fifteen women with HM attending the Department of Obstetrics and Gynecology at Hiroshima University Hospital provided written, informed consent to participate in the study, which was approved by the Research Ethics Committee of Hiroshima University. Samples of DNA, extracted from molar tissue, maternal blood and paternal blood were investigated by quantitative fluorescent PCR (QF-PCR) with short tandem repeat markers specific for loci on each of chromosomes 21, 18, X and Y. Quantitative analysis of the amplification products allowed the diagnosis of the genetic origin of HM. Among 15 samples of HM, DNA from only paternal origin was found in 14 cases(93.3%) and from both parents in one cases (6.7%); and in the former, the homozygous CHM and the heterozygous CHM were 12 cases(80.0%) and 2 cases(13.3%), respectively. Multiplex quantitative fluorescent analyses were performed in about 8 hours from collection of the samples. QF-PCR is useful method for the rapid determination of the genetic origin of the hydatidiform mole.

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Rapid Detection of Chromosomal Aneuploidies in Uncultured Amniocytes by Multiplex Ligation-dependent Probe Amplification (MLPA). *R.J. Sinke[1], J. Meijer[1], I. van de Brug[1], I. Vossebeld-Hoff[1], R. Jansen[1], R.B. van der Luijt[1], G.C.M.L. Page-Christiaens[2], J.M. de Pater[1], J.K. Ploos van Amstel[1], P.F.R. Hochstenbach[1].* 1) Dept. of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 2) Dept. of Perinatology and Gynaecology, University Medical Center Utrecht, Utrecht, The Netherlands.

Trisomy of chromosome 13, 18 and 21 and sex chromosome aneuploidy account for 60-80% of abnormal fetal karyotypes detected in cultured amniotic fluid cells. Recently, multiplex ligation-dependent probe amplification (MLPA) has emerged as an alternative PCR-based technique for the relative quantification of genomic DNA sequences. To test whether multiplex ligation-dependent probe amplification (MLPA) can be used for the detection of aneuploidy of chromosomes 13, 18, 21, X, and Y in uncultured amniocytes, the chromosome copy numbers were determined by analysing the relative amount of PCR-product of chromosome-specific MLPA-probes. Results were available within 48 hours and were compared with those of karyotyping. Based on a prospective, clinical study of 527 amniotic fluid samples, we investigated whether MLPA can be used for the detection of common chromosomal aneuploidies in uncultured amniotic fluid cells. There were 517 conclusive MLPA tests. In 514 tests results were concordant with those of karyotyping. There were 2 cases of 69,XXX triploidy that can not be detected by MLPA and there was 1 false positive result. Here, MLPA indicated a 47,XXY fetus whereas the karyotype was 46,XY. We correctly identified all 23 cases of autosomal trisomy and the single case of monosomy-X in samples collected from 16 up to 36 weeks of gestation. In 10 cases (2%) the result was inconclusive due to an insufficient amount of DNA. Sensitivity, specificity and failure rate of MLPA were comparable to those of FISH and QF-PCR. Aneuploidy screening in uncultured amniocytes by MLPA is feasible in a clinical diagnostic setting, yielding an informative and rapid result in 98% of cases.

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The identification of a dicentric X-chromosome in embryos undergoing preimplantation genetic diagnosis (PGD) for aneuploidy screening in a couple with normal karyotypes and eight previous losses. *W.G. Kearns[1], R. Pen[1], J. Kaminsky[1], L. Timmreck[2].* 1) Shady Grove Center for Preimplantation Genetics; 2) Shady Grove Fertility Reproductive Science Center Rockville, Maryland.

In vitro fertilization and PGD for aneuploidy screening was performed on a couple with 8 previous losses. The 38 year-old woman and her 39 year-old husband have normal karyotypes, and he has abnormal semen parameters. Laser assisted embryo biopsy was performed on 6 day-3 cleaving embryos. Cell lysis, fixation, hybridization, and stringency washes were performed according to routine laboratory protocols. Multi-color fluorescence in situ hybridization (FISH) was used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. Five of 6 embryos were aneuploid. Three embryos were aneuploid for 2 different chromosomes, one was abnormal for 3 chromosomes and one embryo was abnormal for 4 separate chromosomes. Two of 6 embryos had X chromosome fluorescent signals consistent for the presence of a dicentric chromosome. Therefore, we used telomere FISH probes to identify telomeric sequences on the putative dicentric X chromosomes. Dicentrics whose centromeres are joined by their q arms will have two p-arm and no q-arm telomeric sequences, whereas dicentrics joined by their p arms will have two q-arm and no p-arm sequences. Both nuclei with a putative dicentric X chromosome showed two p-arm sequences but no q-arm sequences. These dicentric chromosomes are daughter cells from a parental cell containing a q-arm paracentric inversion of one X chromosome. The presence of a dicentric X chromosome in two of six cleaving embryos in a couple with normal somatic cell karyotypes and eight previous losses suggests possible gonadal mosaicism for an X-chromosome with a q-arm paracentric inversion. When a day-3 embryo includes a dicentric X chromosome with two active centromeres, subsequent cell divisions may result in chromosome breakage and the loss of the putative dicentric X chromosome. This results in cells with just a Y chromosome and subsequent lethality or embryos with Turner or mosaic Turner syndrome and likely miscarriages.

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Deletion 2q37.3 in a prenatal case followed for over 5-years: Review of the literature to trace the natural clinical history for genetic counseling and clinical monitoring. *C. Atzinger[1], C. Greene[1], K. Reddy[2].* 1) Department of Clinical Genetics, Univ of Maryland, Baltimore, MD; 2) US Labs, Irvine, CA.

A 20-year-old woman had amniocentesis because of abnormal maternal serum triple marker screen. The fetus was found to have an apparent de novo deletion of 2q37.3 by cytogenetics and fluorescence in situ hybridization (FISH) and the fetal ultrasound was normal. Following counseling the couple decided to continue the pregnancy. The clinical course of the child was followed over the next five years. At 5-years of age the proband shows developmental delay, behavioral problems including hyperactivity. The physical findings were alopecia, eczema, and brachydactyly. The natural history of our proband and other reported cases was contrasted. This detailed clinical comparison [in an excel worksheet] will provide additional information useful in genetic counseling and serve as a clinical monitoring tool when 2q37 deletion is detected.

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Subtelomeric FISH in abnormal offspring reveals familial cryptic translocations in two couples with habitual abortions and normal G-banded chromosomes. *E. Zackai, N. Unanue, D.M. McDonald-McGinn, K.L. Ciprero, S. Purandare, N. Sondheimer, S. Saitta, D.A. Driscoll, B.S. Emanuel, N.B. Spinner.* Division of Human Genetics, The Children's Hospital Philadelphia, Philadelphia, PA.

Chromosome analysis in couples with habitual abortions has been useful in diagnosing translocation carriers and has become standard of care. In contrast the yield is low in series of couples with habitual abortion tested with subtelomeric FISH (Fan and Zhang AJMG 109:154, 2002; Jalal et al. Genet in Med 5:28, 2003; Benzacken et al. Hum Reproduct 17:1154, 2002). Here we discuss two couples with histories of multiple miscarriages who had normal conventional G-banded chromosome analyses prior to the birth of an affected child. Both couples had normal prenatal chromosomal analysis of the affected fetuses. One amnio was performed because of multiple fetal anomalies including diaphragmatic hernia and congenital heart disease. Whereas, the second woman underwent CVS due to advanced maternal age. Both neonates had cytogenetic studies at birth. The second because of microcephaly, bicuspid aortic valve and butterfly thoracic vertebrae. Chromosomes were again interpreted as normal. Subtelomeric FISH studies revealed a maternally inherited unbalanced translocation in both cases [46,XY,der(15)t(1;15)(q44;q26.3); 46,XY,der(22)t(19;22)(q13.33;q13.31)]. Upon review of the original karyotypes with conventional G-banding, the chromosomal ends involved in the translocations appear identical under the microscope. Therefore, it is quite plausible that without subtelomeric FISH studies these familial translocations would have remained undetected. The breakpoints are currently being mapped in both cases. We presume them to be proximal to the subtelomeric regions. Of note, regarding Case I, diaphragmatic hernia is known to be mapped to the 15q24-26 region (Slavotinek et al. AJHG 75:150(a733), 2004) whereas in Case II the ARSA gene, which maps to 22q13.31-13.33, is deleted. The importance of developing cost effective methods to detect cryptic translocation carriers presenting with habitual abortions, before the birth of a child with multiple anomalies, will be discussed.

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Thoracoamniotic shunting in a case of fetal Down syndrome with hydrops. *H. Sago[1], S. Hayashi[1], T. Isojima[2], T. Nakamura[2], Y. Itoh[2], M. Kitagawa[1].* 1) Dept Fetal Medicine, Natl Ctr Child Health & Dev, Tokyo, Japan; 2) Dept Neonatology, Natl Ctr Child Health & Dev, Tokyo, Japan.

Fetuses with Down syndrome, a major cause of hydrops fetalis, have a higher rate of spontaneous death than normal fetuses and the prognosis is generally poor. Thoracoamniotic shunting is an appropriate treatment option for fetal hydrops caused by pleural effusion. However, this treatment is not usually applied in the case of Down syndrome. In this report, we describe the successful treatment of a case of fetal Down syndrome with hydrops by pleural effusions by thoracoamniotic shunting. Ultrasonogrphy at 29 weeks gestation revealed fetal skin edema, ascites, bilateral hydrothorax and polyhydramnios. The fetal morphological evaluation and heart rhythm were normal. Immune hydrops or a viral infection was excluded. Bilateral pleural aspiration and amniodrainage were performed. The lymphocyte count of the aspiration was consistent with chylothorax. The effusions reaccumulated and thoracoamniotic shunting using a double-basket catheter were performed bilaterally at 30 weeks gestation. The pleural effusions and hydrops resolved. The fetal karyotype was trisomy 21. An emergency Cesarean section was performed due to premature rupture of the membrane at 33 weeks gestation. A 1410g female neonate with typical facial features of Down syndrome was delivered. The neonate responded well to mechanical ventilation and thoracic drainers that were inserted for a month, and made a gradual transition from parental to intestinal nutrition. Currently, aged 8 months, she was as well as a Down syndrome child without cardiac defects. We therefore conclude that thoracoamniotic shunting can be used as a treatment option for fetal Down syndrome with pleural effusions.

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Quantification of palindrome-mediated de novo translocations in human sperms by multi-PCR. *J. Li-Ling[1], T. Wen[1], X. Ji[2], J. Zhang[1], Y. Zhao[1].* 1) Department of Medical Genetics, China Medical University, Shenyang 110001, China; 2) Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming 650091, China.

Following the confirmation that the most common human non-Robertsonian translocation, t(11;22)(q23;q11), is mediated by palindromic sequences from relevant genomic regions, researchers have recently discovered several other translocations by similar mechanism, including t(1;22)(p21.2;q11.21), t(4;22)(q35.1,q11.2), t(17;22)(q11;q11) and t(x;22)(q27;q11.21). As discovered by Kurahashi and Emanuel (2001), unexpectedly high rate of de novo t(11;22) translocations occur in sperms from normal males. For those with more mutations are at a higher risk to produce offspring with birth defects and mental disabilities, we explored to quantify common palindrome-mediated translocations in sperm samples coming from genetic counseling. Based on the results of BLAST by palindromic sequence from 22q11 LCR22-B, multi-PCR primers were designed by using Primer 5 or derived directly from the literature, which included five known translocations and three predicted ones, i.e., t(5;22)(5q33.3;q11), t(9;22)(p13.1;q11) and t(16;22)(p12.3;q11) (Homo sapiens clones RP11-342F17, RP11-271O3 and RP11-295M3). Nested PCR was used to increase the sensitivity of detection. Quantification of the mutations was achieved by repeat measurement, as described by Kurahashi and Emanuel. Results of PCR were confirmed with position of electrophoretic bands with predicted lengths as well as direct sequencing. So far we have found that, in addition to t(11;22), several other translocations, including t(1;22), t(15;22), t(17;22) and t(x;22) are also common in sperm samples from normal males as well as oligospermic patients. The overall mutation rate varied significantly. t(4;22), t(5;22) and t(9;22) were only occasionally seen. However, it seems too early to decide whether similarity between palindromes may have an influence on the rate of relevant translocations. Simultaneous measurement of 5 most common translocations based on multi-PCR is underway. Real-time PCR is also being tried, which may help to simplify the test.

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Post-natal follow-up of prenatally diagnosed trisomy 16 mosaicism. *S. Langlois[1], P.J. Yong[1], S.L. Yong[1], D. Kalousek[1], P. Miny[2], W.P. Robinson[1].* 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Abt Medizinische Genetik, Universitatskinderklinik, Basel Switzerland.

Pregnancies diagnosed with trisomy 16 (T16) mosaicism by either chorionic villus sampling (CVS) or amniotic fluid testing (AF) are known to be at significant risk of intrauterine growth restriction, fetal malformation (especially septal defects of the heart), maternal preeclampsia, and intrauterine or neonatal death. However, there is little data on the long-term prognosis for these infants in terms of their health and development. We report on the outcomes of 30 pregnancies diagnosed prenatally with T16 mosaicism where data on children greater than 1 year (ages 1-13 years) was available. Postnatal outcome data was collected through completion of a questionnaire, direct clinical evaluation or through review of published data. Sixteen cases were diagnosed with trisomy 16 on CVS with normal follow up AF in 15 cases and no AF performed in one case. Of these cases, growth parameters at birth were below - 2SD for length in 3/11 cases and for weight in 4/10 cases. Catch up growth was seen for length in all 3 cases and for weight in 2 of the 4 cases. In terms of development, no cases of mosaicism detected only on CVS had global developmental delay, and only one case had speech delay. In fourteen cases, mosaicism for T16 was diagnosed on AF. Growth parameters at birth were below - 2SD for length in 5/8 and for weight in 6/7 cases and catch-up growth was seen in all cases. Amongst the AF detected cases, 4/14 cases had global developmental delay. The risk of developmental delay was also associated with the presence of major and minor malformations: 4/9 cases with anomalies had associated developmental delay compared to 0/5 cases with no abnormalities. Uniparental disomy was present in 8 cases diagnosed by CVS and 5 cases diagnosed at AF but was not associated with adverse longterm outcome. In conclusion, the majority of prenatally diagnosed T16 mosaic cases have a good outcome. However the finding of mosaicism on amniocentesis plus major/minor abnormalities is associated with an increased risk of developmental delay.

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A new molecular method to measure the copy numbers of chromosomes or genes. *K.M. Hong[1], M.H. Ko[1], Y.B. Choi[1], N.H. Kim[2], I.H. Kim[1], Y.J. Kim[1].* 1) Research Institute, National Cancer Center, Goyang, Gyeongggi-do, Korea; 2) Department of Internal Medicine, Wonkwang University College of Medicine, Iksan, Korea.

Karyotyping is currently the gold standard for the diagnosis of chromosomal aneuploidies. Karyotyping takes about two weeks and needs qualified technicians, so several molecular techniques were recently introduced. The relative amplification during PCR was stabilized with the introduction of MLPA (multiplex ligation-dependent probe amplification). However, the standard deviation values from MLPA are relatively big (0.13-0.3). Here we introduce a new molecular method to measure the copy numbers with the SD values of 0.02-0.05. For the detection of trisomy 21 and 18, the relative ratios of three genes (*DSCR1* for trisomy 21, *.DCC* for trisomy 18 and hexokinase gene for control) were tested by our new method. All the ratios from about 30 control samples were within 0.9-1.1 after normalization. All the ratios from 10 Down syndrome samples were in the trisomy category (two normalized ratios were over 1.4). This molecular assay takes about 3-4 hours to get the final results when the genomic DNA is ready and is easily expandable to dozens without changing the total assay time. This method can be used for the screening of chromosomal anomalies replacing the FISH (fluorescence *in situ* hybridization) before reporting the results with karyotyping and for the detection of exonal deletions in inherited diseases including Duchenne muscular dystrophy. Also this method can be used for the detection of chromosomal changes in cancer samples, and for finding new candidate gene deletions or amplifications.

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Improved second trimester prenatal screening for Down syndrome: Addition of invasive trophoblast antigen (ITA). *C. Strom, P. Peterson, E. Carlton, P. Vendeley, J. Neidich, R. Pandian.* Med Dir, Genetics Testing Ctr, Nichols Inst/Quest Diagnostics, San Juan, Capis, CA.

ITA, formerly known as hyperglycosylated hCG (hhCG), is elevated in Down Syndrome (DS)-affected pregnancies. Recent studies have shown that ITA is a useful marker for maternal serum prenatal screening. Based on archived case control material, Palomaki, et al. (Clin Chem. 2004) predicted addition of ITA to AFP, hCG, uE3, and inhibin A (DIA) would result in a DS detection rate of 83% in the second trimester, up from 72% (triple screen) and 79% (quad screen). We subsequently developed a risk assessment algorithm that included all 5 markers and used it to determine the DS detection rate in a prospective study involving 1480 pregnancies, 19 of which were DS-affected. This penta screen detected 16 of the 19 (84%) cases as predicted. Since screening programs use fixed odds ratios (usually 1:270 - 1:300) as the cut-off between positive and negative screens, we would expect the screen positive rate to decrease as the programs detection rate improves. To test this hypothesis, we have analyzed data from screening programs that use the triple (n=3764), quad (n=3441), and penta screens (n=1967). For women under the age of 35, the screen positive rate was 5.0%, 4.6%, and 3.6% in the triple, quad, and penta screens, respectively. For women over the age of 35, the screen positive rate was similar in the triple and quad screens (22%) but lower in the penta screen (16.7%). Thus, the penta screen appears to increase the Down syndrome detection rate while decreasing the screen positive rate. Theoretically, this should lead to a decrease in the number of amniocenteses performed for each affected fetus detected.

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Frequencies of chromosomal abnormalities at amniocentesis: over 4 years of cytogenetic analyses. *M. Kasap, O. Demirhan, A. Pazarbasi, D. Suleymanova-Karahan, E. Tunc, D. Tastemir.* Medical Biology and Genetics, University of Cukurova, Adana, Turkey.

Invasive methods in prenatal diagnosis has been recognized as safe reliable methods for couples at high risk of giving birth to child with chromosomal abnormalities. Amniocentesis is an important prenatal diagnostic technique offered to pregnant women at increased risk of chromosome abnormalities. It is usually performed between 15 and 17 weeks of gestation and is the most widely used invasive technique. Here, we have reported the 2660 amniocentesis results from Department of Medical Biology and Genetics, Fakulty of Medicine, University of Çukurova between March 2001 and May 2005. The total abnormal karyotype rate was found 4.3% (114/ 2660). Among these abnormal karyotypes, the frequency of numerical and structural chromosomal abnormalities were 58% (66/114 case) and 42 % (48/114 case), respectively. In the numerical abnormalities, the frequency of trisomy 21 was the highest 25% (28/114), followed by sex chromosome aneuploidies in 16 cases (14%). In the structural chromosomal rearrangements, 25 cases had familial inversions on chromosome 9 (22%). Chromosomal deletions in 4 case (3.5%), translocations in 11 cases (10%) and duplication in two case (2%) were detected. The maternal age was the most common indication (44.6%), followed by abnormal serum levels of screening test results (38.6%), abnormal ultrasound screening results (6.1%), parental chromosome aberrations (4.2%), previous fetus/child with chromosome abnormalities (2.3%). Maternal age ranged from 18 to 49 years (Mean: 32.6 years) and mean of gestational age was 19 weeks. The 51% and 49% of the total karyotypes were male and female, respectively.

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Relationship between gestational age and fetal karyotype in levels of cell-free fetal DNA in amniotic fluid. *O. Lapaire[1], K.L. Johnson[1], H. Stroh[1], I. Peter[2], J.M. Cowan[1], U. Tantravahi[3], B. OBrien[1], D.W. Bianchi[1].* 1) Division of Genetics, Tufts University School of Medicine, Boston, MA; 2) Institute of Clinical Research and Health Policy Studies, Tufts University, Boston, MA; 3) Department of Pathology, Women and Infants Hospital, Providence, RI.

Amniotic fluid (AF) is a promising source of cell-free fetal DNA (cffDNA), with an approximately 100-200 fold greater concentration compared to maternal plasma. However, its potential role in non-invasive prenatal diagnosis has been rarely studied. Residual AF supernatant samples (5.5-10 mL) were obtained from 25 euploid, 13 trisomy 21 and 5 trisomy 18 fetuses, collected after clinically indicated amniocenteses. Median gestational age (GA) at amniocentesis was 16.85 weeks (16.43; 18.14). DNA was isolated from AF samples using the DNA Blood and Body Fluid Protocol (Qiagen, Inc.) and quantified using real-time PCR (Applied Biosystems, Inc.). The median amount of GAPDH DNA detected from all samples was 1515 genome equivalents/mL (1091; 3903). A significant positive correlation was observed between cffDNA levels of euploid fetuses and GA (r=0.71) as well as between trisomy 18 fetuses and GA (r=0.82). In contrast, no correlation was observed between cffDNA from trisomy 21 fetuses and GA (r= -0.03). We speculate that differences in AF cffDNA levels between euploid and some aneuploid fetuses may be due to tissue-specific kinetic mechanisms (e.g. DNA release and/or clearance) or developmental abnormalities. These results differ from those obtained from cffDNA isolated from maternal plasma/serum, where cffDNA levels increase with GA in both euploid and aneuploid fetuses, which may be due to the origin of cffDNA (e.g. fetal organs that come in direct contact with amniotic fluid such as lung and kidney versus placental or circulatory cells).

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Prenatal karyotypes from different organic material besides the traditional ones. *R. Gus[1, 2], M.T.V. Sanseverino[1, 2], J.A.A. Magalhães[2], O.A. Magalhães[2], R. Giugliani[1].* 1) Genetics Department, Hospital de Clinicas de Porto Alegre; 2) Fetal Medicine Group, Hospital de Clinicas de Porto Alegre, RS, Brazil.

The study of fetal karyotype became, in the last decades, a very important tool for either genetic counseling or fetal diagnosis itself. In our public hospital we offer such service since 1989, being a very unique situation in Brazil. Nowadays, at our laboratory, fetal health can be detected at three different levels: biochemical, molecular and chromosomal. Also, with the development of the ultrasound technology, many fetal malformations can be detected; therefore, specific studies can be done. The objective of this study was to describe the experience of the Fetal Medicine Service at Hospital de Clinicas de Porto Alegre, South Brazil, from December 1989 to December 2003. Here, we emphasize the utilization of different fetal materials for karyotyping, such as: amniotic fluid, chorionic villus sampling, blood, urine, intraperitoneal fluid, cerebro-spinal fluid and fluid from kidney cysts. During the period described above, 2021 exams were done, and the patients had consultation of genetic counseling before and after the results. Amniocentesis were done in 1638 patients, 320 chorionic villus sampling, 54 cordocentesis, 4 bladder punctures and from hydronephrosis, 2 intraperitoneal fluid collection, 1 puncture collection from multicystic dysplastic kidney, 2 punctures from lymphatic fluid in cystic hygromas. Those last 9 cases, with uncommon materials, were collected because of severe oligohydramnios. From the total of 2021 exams, 1995 were successful (98.71%), and only 26 cases (1.29%) had no results, 18 of them failed during the set up of the cytogenetic laboratory. The failed cultures referred to amniotic fluid or chorionic villus sampling. All the other cultures, including the uncommon materials, had their culture grown with success, achieving the desired results. With these results we are able to demonstrate that sometimes it is necessary to collect others materials from the fetus (like severe cases of oligohydramnios), besides the traditional ones. These materials can be an excellent alternative for karyotyping, sometimes with better results.

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Tay Sachs screening in HIV positive pregnant women. *A.M. Roe, G. Zapantis, S. Nakagawa, S.J. Gross.* Obstetrics and Gynecology, Albert Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY.

Background: Pre-conceptual carrier testing for Tay Sachs disease (TS) is offered to patients with a family history of TS and individuals of Ashkenazi Jewish (AJ) or French Canadian descent. Carrier testing for TS is currently performed by examining the activity of -hexosaminidase A (Hex A) in serum, leukocytes (wbc), or platelets. Approximately 7000 pregnant women in US have HIV and due to great strides in therapy it is now regarded as a chronic infectious process. We present a case of a woman who was HIV positive and underwent prenatal wbc screening for TS with ambiguous results. The benefit of platelet assay in chronic illness is addressed. **Case Study:** A patient of half AJ and half African American descent underwent TS carrier screening during an evaluation for tubal factor infertility and was found to have an "ambiguous" result of 62% on wbc Hex A activity (normal range 63-75%). She was HIV positive and was taking nevirapine and zidovudine/lamividine. Her viral load was 50 and her wbc count was 7.0 thousand/cu mm with a normal differential (62.9% neutrophils, 30% lymphocytes, 5.8% monocytes, 0.9% eosinophils, 0.3% basophils). She had no other medical problems. Molecular genetic testing of the three most common AJ mutations was negative. Measurement of platelet Hex A activity was performed and was normal (62%, 64%).

Discussion: The wbc assay for TS is a common and reliable testing method in pregnancy. However, disorders that can affect wbc count or function may alter results and lead to ambiguous findings. HIV disease has been associated with an increase in the plasma activity of Hex A and with an increase in the percentage of the Hex I fraction. Leukocytes are a mixture of cell types and higher levels of Hex A are found in granulocytes, therefore, the range of error is greater in the wbc assay than in specific tissue or cell types such as platelets. Furthermore, as of yet, there is limited data regarding anti-retroviral medications and Hex A activity. We recommend that HIV in pregnancy be regarded as a chronic infectious process and that the platelet assay modality may be a better choice for such circumstances.

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Current opinions about the use of X-linked carrier embryos in preimplantation genetic diagnosis. *A. Perez[1], L. Demmer[2], J. Jonas[2], N. McIntosh[1].* 1) Biology, Brandeis University, Waltham, MA; 2) Pediatrics, Tufts-New England Medical Center, Boston, MA.

Use of carrier embryos of X-linked conditions (CE-XLC) in preimplantation genetic diagnosis (PGD) is controversial. Using such embryos may produce females who have health concerns related to their carrier status, and such women will also be faced with reproductive decisions related to the X-linked condition when they start their families. On the other hand, excluding the use of CE-XLC may be unethical because most resulting individuals would be healthy. Currently no guidelines exist regarding the use of CE-XLC in PGD. The purpose of this study was to survey providers and consumers/potential consumers about their views regarding the use of CE-XLC in PGD. 143 physician providers of PGD identifited from PGD websites were invited via email to participate in an anonymous online survey consisting of 22 questions regarding demographic information, experience with, and attitudes toward PGD. Carriers of X-linked conditions were invited through 11 online support groups to participate in a similar anonymous online survey consisting of 24 questions related to their experiences and attitudes with being X-linked carriers, potential symptoms, reproductive planning, PGD, use of carrier embryos, need for regulation of PGD, as well as demographics. 32 providers and 116 consumers completed surveys. Results show that while most providers (61%) felt that CE-XLC should be used in PGD, potential consumers of PGD were divided in their opinions (43% Yes, 38% No, 19% unsure). The majority of providers (57%) also felt that the US should not adopt guidelines for the use of PGD, versus 70% of potential consumers who felt that guidelines might be beneficial. Of consumers who had undergone PGD (n=4), 50% stated that using CE-XLC was discussed with them. While results suggest differences in opinion regarding the use of CE-XLC, open discussions between consumers and providers may mitigate their differences and facilitate achievement of common goals. In addition, there does not appear to be a consensus between these groups regarding the need for guidelines in this area.

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Genometer, Amniometer and Genetic Ginnie - novel web based programs for comprehensive prenatal individualized information. *M. Shohat, D. Keynan, A. Labrov, N. Harel, G. Snir, M. Shohat, R. Wardenfeld.* Medical Genetics Institute, Rabin Medical Center, Petah Tikva, Israel.

Because the field of medical genetics is expanding, more time is required to explain genetic data to patients. We have developed three different web based programs that cover most of the information required by couples before genetic counseling. 1. Genometer: A self-completed computerized program that calculates the couples carrier risk for each of 22 diseases offered by the genetic services in Israel. The program can be easily adjusted for any country/populations by adding the relevant diseases, ethnic groups and carrier frequencies in each group. Carried out prior to or in early pregnancy and based on the ethnic origin of the couple, it provides a list of diseases based on the calculated combined ethnic risks. It is offered before referring couples for genetic screening. 2. Amniometer: A program to assist the physician with normal cases (where the results of all the routine tests performed during the pregnancy are normal and there are no other issues that require genetic counseling) in the decision-making whether or not to perform amniocentesis. The program combines all the different risks and depicts the final risk on a scale that can be easily understood. If there are any abnormalities the couple can enter more information in advance of genetic counseling. The geneticist can control the program easily. 3. Genetic Ginnie: A complete genetic questionnaire for couples with a positive family history or abnormal findings. In addition to an encyclopedia the program asks all the standard questions - some will appear only if the person gives specific answers. The report is long, written for lay people, and allows them to obtain additional relevant information. It saves time during the genetic counseling session and is intended for couples before they come to the geneticist. All these programs have been tested and are helpful both to the staff and the couples. Based on analysis by geneticists as well as on a general random telephone survey, it was concluded that this individualized genetic information provides a real professional need to the public.

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A new personalized genetic information program for carrier screening - acceptance by the population screened.

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Over the last 10 years tests for carrier screening for 22 common genetic diseases have been introduced and offered to the Jewish population in Israel, according to the ethnic origins of the couples. This has caused confusion among both public and physicians regarding which tests to recommend to each couple. In order to assist couples in their decision, a major HMO in Israel has analyzed the impact of a new strategy for personalized genetic information for carrier screening. The service is based on a self-completed computerized program (Genometer), which calculates the couples carrier risk for each of the 22 diseases. Based on their frequency and severity, the diseases are ranked in 4 categories: 1. Recommended tests (e.g. Ataxia Telangiectasia in North African Jews) 2. Tests for other severe diseases that should be considered (e.g. Bloom syndrome in Ashkenazim) 3. Tests for common but mild/treatable diseases (e.g. Gaucher disease in Ashkenazim) 4. Tests not relevant to the couples ethnic origin (e.g. cystic fibrosis in Iraqi Jews). The computerized program is web based and can be easily updated. 120 trained Ob/Gyn nurses have been recruited to offer the program to couples prior to or in early pregnancy in 90 countrywide clinics. The tests are self-paid by the couples ($25 per disease). Since its initiation over 1000 women have been using the program each month. A telephone survey was carried out among 300 who were asked about different parameters regarding its benefits. 90% said they were very satisfied with the information provided, 83% recommended the service to their pregnant friends, and 67% underwent the genetic tests following the information session. The program has been tested and approved by geneticists and genetic counselors in Israel, who found that the personalized information was accurate and there were no errors in the categorization of the diseases/tests to the different recommendation groups. It was concluded that this program provides a real professional need to the public.

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Congenital nephrosis in the offspring of two consanguineous couples from the same Afghani family. *F.H. Morris[1], J. Taylor[1], J. Shields[2], D.B. Rogers[1].* 1) Genzyme Genetics, Orange, CA; 2) Valley Perinatal Medical Group, Tarzana, CA.

Case A: A 27-year-old G2P1 Afghani woman presented at 17 weeks gestation (GA) because of an elevated maternal serum alphafetoprotein (MSAFP) on an Expanded AFP test. The patient and her spouse were first cousins once removed. A fetal ultrasound was normal but amniocentesis revealed a 46,XY fetal karyotype with amniotic fluid AFP (AF-AFP) elevated at 26.45 multiples of the median (MoM) and negative acetylcholinesterase (AChE). Repeat amniocentesis yielded an AF-AFP of 29.97 MoM and negative AChE. After genetic counseling the patient elected to continue her pregnancy and delivered a normal-appearing newborn at term who was discharged home after only two days. Several days later the infant required acute hospitalization and was subsequently diagnosed with congenital nephrosis.

Case B: Eighteen months after Case A presented, a 31-year-old G3P1SAB1 Afghani woman with a different last name was seen at 18 weeks GA due to an elevated MSAFP. The patient stated that she and her spouse are second cousins through her father and first cousins once removed through her mother. She also has a nephew who needs a kidney transplant. Following a normal ultrasound, amniocentesis revealed a 46,XY karyotype, AF-AFP = 31.14 MoM, and negative AChE. Repeat amniocentesis found an AF-AFP = 41.52 MoM. The patient terminated the pregnancy. Molecular analysis of cultured amniocytes found an amino acid change of uncertain significance in the Finnish Congenital Nephrosis (NPHS1) gene. The variant appears to be present in a homozygous state.

Our patients were confirmed to be from the same family, each having married the others brother! The multiple layers of consanguinity between these couples are quite complex, but the variant detected in Case B likely represents a disease-causing mutation in the NPHS1 gene that has also caused congenital nephrosis in Case As son. The variants significance will be confirmed via subsequent molecular analyses of both couples and the affected child. Effective early prenatal diagnosis should then be possible by screening for the variant in future pregnancies.

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Is Pallister-Killian Syndrome associated with advanced maternal age? A review of the Genzyme Genetics Database. *R.L. Arvon[1], G. Bega[1], J. Pelham[2], S. Weiner[1].* 1) Dept OB/GYN, Thomas Jefferson Univ, Philadelphia, PA; 2) Washington Hospital Center Washington DC.

Our aim was to investigate the relationship of Pallister-Killian Syndrome (PKS) and advanced maternal age (AMA - maternal age over 35 years at delivery) by reviewing the Genzyme Genetics database for cases of Pallister-Killian Syndrome and/or Tetrasomy 12p. Pallister-Killian syndrome is a very rare syndrome that can be diagnosed prenatally. It is due to a maternally derived isochromosome (12 p10). The Genzyme Genetics data base of invasive prenatal procedures was queried from January of 1995 to December of 2004 for PKS and/or Tetrasomy 12p. A total of 20 cases were identified out of 608,792 reported invasive procedures, all by amniocentesis and not by CVS (known to miss mosaicism). The overall incidence in the second trimester of PKS in our population was 1 in 30,300. Of these, 13/20 were AMA with an incidence of 1 in 47,000. In comparison, the incidence in women under the age of 35 was almost half at 1 in 87,000. The most common reason found for invasive testing was abnormal ultrasound finding and the most common finding reported on the ultrasound was congenital diaphragmatic hernia (CDH) and then increased nuchal translucency (NT).

	AMA only	Abn. u/s only	AMA/Abn. u/s	Abn serum	AMA/Abn serum
Over 35 yrs.	5	1	4	0	3
Under 35 yrs.	0	5	0	2	0
Total	5	6	4	2	3

Conclusion: This study confirms that PKS is more common in women 35 and over. Women of advanced maternal age who originally declined invasive testing and found to have CDH on ultrasound should be offered karyotype and interphase FISH to rule of PKS.

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Evaluation of Genetic Variation in the Fetus as Risk Factors for Preterm Delivery. *D. Caprau[1], M. Marazita[2], M. Cooper[2], J. Dagle[1], C. Zimmerman[1], K. Johnson[1], K. Orr[1], J. Murray[1].* 1) Univ Iowa, Dept Pediatrics, Iowa City, Iowa; 2) Univ Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

Preterm delivery currently affects more than 400,000 infants born annually in the US and more than four million world-wide. Mortality of these infants remains substantial and morbidities which include chronic lung disease, intra-cranial hemorrhage, patent ductus arteriosus, retinopathy of prematurity, cognitive delay, cerebral palsy, and others remains substantial despite dramatic improvements in both treatment and prevention. Preterm labor resulting in delivery of a premature infant is a complex problem whose etiology likely encompasses both genetic and environmental effects. Genetic risks for prematurity have been evaluated using candidate gene approaches but have focused primarily on the mother rather than the fetus as the risk factor. In this study we undertook a comprehensive examination using cases defined by preterm delivery in an infant coupled with DNA samples from the parents to determine whether fetal genetic variation could be one component of the stimulus to preterm delivery. A total of 490 premature babies with gestational ages between 22-36 weeks (mean= 31.2 +/- 3.6 weeks) and their parents have been enrolled in this study. DNA was extracted from cord blood for the babies and from venous blood or buccal swabs for the parents. Genotyping for all other markers was performed using the TaqMan chemistry as designed by Applied Biosystems. We performed TDT analysis using FBAT approach to assess allelic association with preterm birth. Thirty-seven genes and 90 SNPs were investigated. Significant results have been identified for several genes: Factor 5 (p=0.015), OPRM1 (p=0.021), MTHFR (p=0.043), MTRR (p=0.02) and others. No evidence of fetal risks was associated with IL 6, TNF- or HLA-B or G as had been reported in previous studies. The methods employed here can be extended to additional genes as well as to a similar approach using the mother as a risk case and eventually to look at interactions between mother and fetus. Genes identified in this study should now be evaluated more thoroughly using functional studies and additional genetic variations.

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Genetic study of Sirenomelia, exclusion of major genes (HLXB9, SHH, Patched...) in human sirenomelia. *M. Gerard[1], A. Bourillon[2], M. Sinico[3], C. Touboul[4], JM. Levaillant[4], T. Costa[5], Y. Roumazeilles[6], B. Haddad[4], A. Boutron[7], A. Bazin[8], J. Martinovic[8], F. Encha-Razavi[3], B. Gerard[2].* 1) Medical genetics, CHIC, Creteil, France; 2) Molecular genetics, Bichat, France; 3) Fetal pathology, CHIC, Creteil, France; 4) Obstetrics, CHIC, Creteil, France; 5) Medical genetics service, Sainte Justine Hospital, Montreal, Canada; 6) Website designer, Paris, France; 7) Fetal cell bank, Kremlin-Bicêtre, France; 8) Pasteur-CERBA laboratory, Pontoise, France.

Sirenomelia is a rare fatal condition characterized by fusion of the lower extremities, and genito-urinary malformations. Sirenomelia is usually sporadic, and monozygotic twins are usually discordant for this malformation (Stocker, 1987). Nevertheless, there are strong evidences for genetic predisposition in sirenomelia (i) reports showed a familial recurrence of sirenomelia (Rudd et al., 1990), or sacral anomaly in a sister of a child afflicted with sirenomelia (Costa, personal communication) (ii) minor defects such as imperforate anus, anomaly of the lumbar spine or renal dysplasia are frequently observed in the family (Rudd et al. 1990; Selig et al., 1993; Akbiyik et al., 2000) (iii) srn mice strain showed 12 percent of recurrence (Orr and al., 1982), (iv) the occurrence of sirenomelia in different mating of mutants mice for Brachyury (T gene), Fused, t and u (Gluecksohn and Dunn, 1942). Thus, sirenomelia could be considered as a complex genetic disease, with possibly a inherited predisposing mutation of one of the several genes essential for the caudal mesoderm induction and additional somatic mutations affecting either the second allele of the inherited mutated allele or others genetic loci (multihit hypothesis). Here, we report the first human genetic study of sirenomelia, on fetuses and sib. Molecular studies were done on HLXB9 gene (Currarino triad), with sequencing of the coding sequence and search for heterozygous deletions using real time PCR, sequencing of the coding sequence of SHH and PTCH. Extension of the study is underway, on the Brachyury gene and the receptor of retinoic acid, CYP26A1 (international collaborative study on the website : www.sirenomelia.org).

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Prenatal diagnosis for Fragile X syndrome using methylation-specific PCR. *P. Limprasert, C. Charalsawadi, T. Sripo.* Human Gen/Dept Pathology, Medicine, Prince Songkla Univ, Songkhla, Thailand.

Fragile X syndrome (FXS) is the most common X-linked mental retardation. It is caused by CGG-repeat expansion within the *FMR1* gene. Affected individuals (full mutation, FM) have greater than 200 CGG repeats, correlated with methylation of the gene. The standard diagnoses are the determination of CGG repeats by PCR (CGG-PCR) and methylation status by southern blot (SB). Methylation-specific PCR (MS-PCR) has been developed for diagnosis of methylation in FXS. We used the MS-PCR protocols of Weinhäusel and Haas *(Hum Genet 2001;108:450-458)* for males and Zhou et al *(J Med Genet 2004;41:e45)* for females. We tested MS-PCR in 25 postnatal FM-FXS cases and 55 controls to verify the protocols in our setting. All results corresponded to the CGG-PCR or SB. We herein report the first time for prenatal diagnosis (PND) for FXS using these MS-PCR protocols. Three FXS female carriers were referred for PND. Amniocentesis was performed at 16-18 weeks gestational age. Fetal sex was determined by using PCR on the *SRY* gene. MS-PCR revealed a full mutation male and a normal male corresponding to the results from the standard diagnoses. Although it is difficult to use MS-PCR for a female due to random X-inactivation, we could identify a suspected premutation/full mutation female in the last PND case. We confirmed that this case was a full mutation female using SB. The advantages of MS-PCR compared to SB are that it requires only a small amount of DNA, and it is less time consuming and less expensive. Integration of MS-PCR into the current practice might reduce the number of cases which require SB, particularly in known FXS families.

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HEXA gene as a model of single cell genetic testing: credibility, precision, implications. *K. Dotan, B. Goldman, B. Feldman, G. Barkai, L. Peleg.* Human Genetics, Sheba Medical Center, Ramat Gan, Israel.

Tay Sachs disease (TSD) is an autosomal recessive disorder due to impair activity of hexosaminidase A. In its severe form it leads to death during infancy. TSD is frequent among Ashkenazi Jews, with a carrier frequency of 1/29. Two mutations underlie nearly 100% of the infantile form +1278TATC (73% of carriers) and IVS12-1G to C (13%). An adult-onset chronic form, caused by the mutation G805A (4% among carriers) is usually in compound with a common one. Preimplantation genetic diagnosis (PGD) was performed in few cases of couples at risk after terminations of affected pregnancies. Our objective was to measure quantitative aspects of single cell analysis: amplification efficiency and allele-dropout rates in uni, simultaneous (of the two common mutations) and multiplex reactions in: peripheral lymphocytes, fibroblasts (amniotic), mesenchymal (CVS) and blastomeres. A set of nested PCR protocols was designed for the three mutations in different combinations. Each of the protocols can be performed in multiplex with one of two markers flanking the HEXA gene found to be highly polymorphic in the carrier population. Mutations were detected by agarose electrophoresis and two fluorescent computerized genotyping systems. Amplification rate was not significantly different among cell types: 86.3% in lymphocytes (82/95) and 82% in the embryonic cell (64/78). ADO rate was significantly lower (1.3%) in embryonic cells as compared to lymphocytes (12%). Lymphocytes treated with PHA to induce cell-divisions showed a 1.8 fold lower ADO rate than non-dividing lymphocytes. Hypothesis: ADO rate is lower in dividing cells having a less compact nucleus structure.

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Preeclampsia and Cell-Free DNA Levels: An In Vivo Model to Study Placental Damage. *M.L. Tjoa[1], T. Cindrova-Davies[2], K.L. Johnson[1], G.J. Burton[2], D.W. Bianchi[1].* 1) Tufts University, Boston, MA; 2) Cambridge University, UK.

Background: Increased cell-free (CF-) DNA levels are present in the plasma of pregnant women with preeclampsia. The underlying pathophysiology for this is presumed to be increased placental apoptosis due to shallow trophoblast invasion and consequent oxidative stress. An *in vivo* model was developed to study the effects of stress-inducing conditions on placental function and release of CF-DNA.

Study Design: Placentas were cultured under normoxic and two stress-inducing conditions (hypoxia/reoxygenation (H/R) and H_2O_2 supplementation) for 7 (n=4) or 15 hours (hr)(n=6). CF-DNA was isolated from the supernatant and quantified by real-time PCR of the beta-globin gene. The amount of isolated CF-DNA was normalized to the placental weight and expressed as pcg of CF-DNA per mg of tissue.

Results:

Culture Condition	7 hr culture	15 hr culture
Normoxic	8798 (2417)	20110 (3748)
H/R	6849 (1279)	26109 (5191)
H_2O_2	6337 (1872)	29583 (5171)

Mean CF-DNA levels are 2-4 times higher in the 15 hr cultures. At 7 hr, there is no difference in CF-DNA levels between the normoxic and the stress-inducing conditions. After 15 hr, a trend showing elevated levels of CF-DNA in the supernatants of stress-induced cultures compared to the normoxic group is observed.

Discussion: Increased culture time under stress-inducing conditions is needed to observe differences in CF-DNA levels. Increased levels of CF-DNA in the plasma of pregnant women may reflect placental dysfunction caused by oxidative stress.

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Accuracy of Down syndrome risk generated by the integrated prenatal screening. *A. Summers, T. Huang, C. Meier.* Genetics Program, North York General Hosp, Toronto, ON, Canada.

Integrated Prenatal Screening (IPS) was firstly described in 1999. It uses 1st. trimester nuchal translucency (NT) and pregnancy-associated plasma protein A (PAPP-A), and 2nd. trimester alpha fetoprotein (AFP), unconjugated estriol (uE3) and total human chorionic gonadotrophin (hCG) to assign women a single risk of having a baby with Down syndrome (DS). IPS can detect 85% of DS at term with a false positive rate of 1.8%. This study evaluates the accuracy of the risk of DS assigned by IPS based on women who completed 5 or 6-marker IPS at North York General Hospital between 11/1999 and 8/2004. During the study period, 31991 women entered the IPS. 30093 (94.1%) women completed IPS and 1898 (5.9%) dropped out, and were assigned a risk based on 1st. trimester screening. The median maternal age was 32 years. 64 (47 in women who completed 5 or 6-marker IPS) cases of DS were identified. The screening software used for IPS is Alpha. The risk cut-off for DS is risk 1 in 200 at term. All screening markers were converted to multiples of the median (MoM). Sonographer specific medians were used for the calculation of NT MoM. Using an established technique, women were ranked and divided into 9 groups according to their risk estimates. Each risk group covered an appropriate risk range and contained similar numbers of DS case except for the group with the highest risk. The mean estimated risk of each risk group was calculated and compared with the observed birth prevalence of DS of that particular group. As approximately 23% of DS pregnancies will miscarry spontaneously, number of DS cases with positive screen results and diagnosed prenatally were allowed for spontaneous miscarriage. Overall, the estimated risks assigned by IPS were close to the observed prevalence although the observed prevalence was slightly lower than the estimated risk in high risk groups. The slightly over estimated DS risk by IPS could be caused by miscarriage in high risk groups as low PAPP-A, increased NT and second trimester DS risk are associated with spontaneous fetal loss. In conclusion, DS risk estimates assigned by IPS were in good agreement with the observed birth prevalence of DS.

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Free-Beta hCG vs. Total-Beta hCG in First Trimester Down Syndrome Screening. *T. Hallahan, D. Krantz, J. Curtis, V. Macri, J. Macri.* Research Div., NTD Laboratories, Huntington Stat, NY.

We analyzed free-Beta, total-Beta hCG and PAPP-A in 105 cases of Down syndrome and 1133 controls. The regressed median MoM in Down syndrome was substantially higher for free-Beta (1.53, 1.65, 1.79, 1.94 and 2.10) than for total-Beta (0.92, 1.09, 1.28, 1.52 and 1.80) at 9, 10, 11 12 and 13 weeks respectively. False positive and detection rates for the combined protocol including NT and PAPP-A were determined by modeling based on biochemical reference data from the present study and published NT distributions. At a 5% false positive rate NT/ PAPP-A alone achieved 90, 86, 83, 80, and 77% detection while including free-beta increased detection to 92, 91, 89, 88 and 87% at 9, 10, 11, 12 and 13 weeks respectively. Substituting total-Beta for free-Beta hCG reduced detection rates to 90, 87, 84, 85 and 85% respectively. The percent of cases detected by each marker that would have been missed by NT/PAPP-A alone are listed below based on the gestational age at blood draw and NT performed between 11w1d and 13w6d.

NT/PAPP-A	9 Weeks	10 Weeks	11 Weeks	12 Weeks	13 Weeks
+ Total-Beta hCG	2%	9%	4%	24%	33%
+ free-Beta hCG	28%	32%	36%	39%	43%

At a fixed 85% detection rate, the false positive rate with NT/ PAPP-A alone was 2.7, 4.3, 6.5, 9.0 and 11.7% while including free-beta hCG decreased these false positive rates to 1.5, 2.1, 2.8, 3.4 and 3.8% at 9, 10, 11, 12 and 13 weeks, respectively. Substituting total-Beta for free-Beta hCG increased false positive rates to 2.5, 3.7, 4.7, 5.4, and 5.2%, respectively. The data confirm previous studies that total-beta hCG is not a marker prior to 13 weeks and that substitution of free-beta with total-beta hCG at 13 weeks in a combined screening protocol would lead to a reduction in early detection of Down syndrome while increasing the false positive rate.

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CLINICAL APPLICATIONS OF PLASMA CIRCULATING mRNA ANALYSIS IN CASES OF GESTATIONAL TROPHOBLASTIC DISEASE. *K. Miura, H. Masuzaki, S. Miura, T. Ishimaru.* Dept OB/GYN, Nagasaki Univ Sch Medicine, Nagasaki, Japan.

Objective: We tried to apply the measurement of plasma mRNA concentration by real-time RT-PCR as a follow-up test for GTD. Material and Method: Three cases with complete hydatidiform moles (CHM) and a case with choriocarcinoma were included. Blood samples (10 ml) were collected at times, before and after suction evacuation or chemotherapy. Plasma mRNA was extracted and a one-step real-time RT-PCR assay was performed as described by Ng et al. HCG-b mRNA was selected as a tumor marker of GTD and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured as a housekeeping gene. Results: In three cases with complete hydatidiform mole (CHM), the day of 1st evacuation and that of 2nd evacuation were expressed as Day 0 and Day 7, respectively. The level of plasma hCG-ƒÀ mRNA in all three cases fell rapidly to undetectable levels by Day 7, and the corresponding hCG protein levels by an immunoradiometric assay (IRMA) also fell to below 1,000 mIU/ml until 35 days after 1st evacuation. In a case with clinical choriocarcinoma, etoposide and actinomycin D therapy was given on Day 0-3, 15-18, 29-32 and 43-46. Her plasma hCG-b mRNA level showed a decreasing tendency similar to the pattern for hCG protein level, but exhibited a transient increase after the first course of therapy and then decreased to 44 copies/ml. The same transient tendency was detected after all subsequent courses of therapy. The concentration of plasma GAPDH mRNA in all three cases with CHM also increased after both evacuations, and that in a case with choriocarcinoma also increased after therapy with etoposide and actinomycin D. Conclusion: Our results suggest that the measurement of plasma mRNA by real-time quantitative RT-PCR can be used as a non-invasive diagnostic, prognostic and follow-up test for GTD.

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Fetal mustache visualized by 3D ultrasonography: first case report. *D.B. Rogers[1], W. Doany[2].* 1) Genzyme Genetics, Orange, CA; 2) Comprehensive Maternal-Fetal Medicine Consultations, Tarzana, CA.

A 40-year-old G6P0SAB5 woman was evaluated at 16 weeks gestation with transabdominal 2D and 3D fetal ultrasound. The examination revealed a single live fetus of a size consistent with dates and no apparent fetal, cord or placental abnormalities except that the fetal mouth appeared to have a soft-tissue overgrowth in the mid upper lip of no larger than two millimeters. On lateral 2D and 3D views a short, tube-like entity could be seen to protrude from the upper lip. AP views by 3D imaging revealed the overgrowth to be two adjoined, but distinct short rods located in the midline of the upper lip, just anterior to the philtrum. There was no evidence of a cleft. Follow-up ultrasonography at 20 weeks revealed a normal-appearing upper lip with apparent total resolution of the previously seen overgrowth.

In 1998 Bronshtein et al. reported finding 7 out of 3000 consecutive fetuses at 14-16 weeks gestation with a rod-like structure on the upper lip (which they termed mustache). Using 2D ultrasonography, they determined that the mustache consisted of two rod-like structures and were located on both sides of the midline on the upper lip. None of the fetuses had any other structural defects. Follow-up ultrasound examinations of the seven fetuses at 20-22 weeks gestation found in each case that the mustache had disappeared. Postnatal examinations also documented completely normal lips.

The fetal mustache described in our case is consistent with those detected by Bronshtein et al. and is likely to represent a delay in the normal embryological development of the human lip. More specifically, this delay is in the degeneration of the tissue remnants of upper lip fusion. Our 3D visualization of the fetal mustache should assist others who might encounter this rare entity on prenatal ultrasound examination. Normal lip development must be confirmed on follow-up ultrasonography and postnatal examination.

M. Bronshtein, E. Z. Zimmer, H. Offir, & S. Blazer. Ultrasound Obstet Gynecol. 12: 252-253, 1998.

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Preimplantation HLA Typing with Aneuploidy Testing. *S. Rechitsky, T. Sharapova, K. Laziuk, R.S. Ozen, I. Tur-Kaspa, A. Kuliev, Y. Verlinsky.* Reproductive Genetics Institut, Chicago, Il.

We presently performed 756 preimplantation genetic diagnosis (PGD) cases for more than one hundred different single gene disorders, resulting in birth of 244 unaffected children. One quarter of these cycles were performed for preimplantation HLA typing, to establish an HLA compatible donor progeny for siblings with bone marrow disorders requiring stem cell transplantation. Because of the majority of such patients being of advanced reproductive age, aneuploidy testing is currently one of the approaches for improving the patients pregnancy outcomes. Using a standard IVF procedure, oocytes or embryos were tested for causative gene mutations or chromosomal aneuploidy, simultaneously with HLA alleles, selecting and transferring only those unaffected embryos, which were HLA matched to the affected siblings and free of aneuploidy. Of 204 embryos tested for HLA and aneuploidy, only 21 of 36 HLA matched embryos were found to be aneuploidy free and transferred in only 13 (48%) of 27 cycles, resulting in clinical HLA compatible pregnancies in 7 (54%) of these cycles. Overall, 1136 embryos were tested for HLA in 125 cycles, resulting in preselection and transfer of 197 (17.3%) HLA matched embryos in 82 (65.6%) cycles, including 107 (16.4%) also free of genetic disease and 88 (18.6%) only HLA matched, which yielded 23 (28%) clinical pregnancies and birth of 19 healthy HLA compatible children. Despite the controversy of PGD use for HLA typing, the data demonstrate the usefulness of the approach for the at risk couples not only for avoiding the birth of affected children with an inherited disease, but also for having unaffected children who may also be potential HLA matched donors of stem cells for treatment of affected siblings.

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Pre-embryonic diagnosis for Sandhoff disease. *Y. Verlinsky, S. Rechitsky, I. Tur-Kaspa, A. Kuliev.* Dept Molec Genet, Reproductive Genetic Inst, Chicago, IL.

Embryos found to be abnormal during preimplantation genetic diagnosis (PGD) are discarded or analyzed to confirm the diagnosis. Because this is ethically unacceptable in some communities and ethnic groups, we developed a pre-embryonic genetic diagnosis, which is based on the sequential first (PB1) and second polar body (PB2) removal, followed by the culture and transfer of mutation free oocytes, while freezing the pronuclear stage oocytes predicted to contain the mutant gene. This was applied to PGD for Sandhoff disease caused by 16 Kb deletion of hexosaminidase B gene, in a couple with a religious objection to the embryo destruction, even if the embryos are affected. Eighteen oocytes were obtained in a standard IVF protocol, from which PB1s were removed following maturation, 4-5 hours after aspiration. PB2s were then removed 6 hrs after ICSI, and both PB1 and PB2 were tested for 16 Kb deletion, involving exons 1, 2, 3, 4 and 5, simultaneously with 5 linked polymorphic markers D5S1982, D5S1988, D5S2003, D5S349 and D5S1404. Based on the results of PB1 and PB2 analysis, 8 oocytes were predicted to contain 16 Kb deletion and frozen at the pronuclear stage (prior to embryos formation), while the remaining 8 oocytes, predicted to be 16 KB deletion free, were cultured and confirmed to be unaffected using blastomere biopsy at the 8-cell stage. Two of these embryos reaching blastocyst stage were transferred back to patient, resulting in an ongoing unaffected singleton pregnancy, representing the first case of pre-embryonic diagnosis, which opens the prospect of PGD in the communities where no embryo testing and discard is presently acceptable.

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Polar Body biopsy based Preimplantation Genetic Diagnosis (PGD) for N-Acetylglutamate Synthase Deficiency (NAGS), and an analysis of ADO rates. *P. Renbaum[1], B. Brooks[2], E. Margalioth[2], T. Eldar Geva[2], Y. Kaplan[1], E. Levy-Lahad[1], G. Altarescu[1].* 1) Medical Genetics and; 2) IVF Units, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Preimplantation genetic diagnosis (PGD) for a single gene defect is presented for a family where both parents are carriers of a rare metabolic disorder, N-acetylglutamate synthase deficiency, causing progressive neurological deterioration and hyperammonia. The N-acetylglutamate synthase (NAGS) gene, located at 17q21, encodes a mitochondrial enzyme that catalyzes the formation of N-acetylglutamate, an essential component of the urea cycle. We have developed a polar body (PB) based multiplex fluorescent PCR protocol to analyze the 2729insC NAGS mutation and five closely spaced, highly polymorphic microsatellite markers D17S902, D17S1861, D17S1868, D17S965 and D17S791 that were informative in this family. Polar bodies 1 and 2 were sequentially biopsied for PGD diagnosis. Of sixteen PB1s analyzed, 13 were heterozygote, one carried the wild type allele, one was inconclusive, and one presented total amplification failure. Heterozygote PB1s are the result of crossover in meiosis and enable the identification of Allele Drop Out (ADO) in PCR analysis. The 13 heterozygote PB1s biopsied were each analyzed with six informative assays for a total of 78 individual reactions. We observed ADO in 17 reactions, resulting in an allele drop out rate of 22%. Of the Sixteen PB1s analyzed, eight of these fertilized, extruded PB2s and developed into 6-8 cell embryos on day 3. Of these, PB1 and 2 analysis showed that three oocytes carried the wild type copy of the NAGS allele and all three relevant embryos were transferred to the mother precluding the need to perform blastomere biopsies. This single cycle of PGD resulted in an ongoing twin pregnancy. The occurrence of ADO in single cell analysis is the primary cause of misdiagnosis in PGD, and the main reason for including polymorphic markers in the analysis. The high rate of ADO seen in PB1s emphasizes the necessity of using polymorphic markers in PGD in order to prevent misdiagnosis. To our knowledge this is the first report of PGD for this rare metabolic disorder.

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Preimplantation genetic diagnosis for tuberous sclerosis. *P. Burlet[1], N. Frydman[2], N. Gigarel[1], V. Kerbrat[3], S. Lebon[1], JP. Bonnefont[1], E. Feyereisen[3], G. Tachdjian[2], R. Frydman[3], A. Munnich[1], J. Steffann[1].* 1) Service de génétique, U393, Hopital Necker Enfants-Malades, Paris, France; 2) Service de biologie de la reproduction, Hôpital Antoine Béclère, Clamart, France; 3) Service de gynécologie-obstétrique, Hôpital Antoine Béclère, Clamart, France.

Tuberous sclerosis (TSC) is a frequent autosomal dominant condition caused by various mutations in either the hamartin (TSC1) or the tuberin gene (TSC2). Disability in TSC patients most often results from brain involvment, including seizures and mental retardation. However, variable expressivity of the symptoms precludes any assumption as to the clinical outcome of an affected foetus, and makes genetic counselling difficult. For some couples, the only acceptable manner to ensure the birth of an unaffected child consists in preimplantation genetic diagnosis (PGD), an alternative to prenatal diagnosis because it circumvents termination of pregnancy. PGD was offered to an at risk couple owing to a paternal c.737+1G>A mutation in the intron 8 donor site of the TSC1 gene. The single-cell test was based on multiplex fluorescent PCR amplifiying simultaneously TSC1 exon 8 and a closely linked microsatellite marker (D9S1830), followed by MboII restriction analysis for the mutation. Single-cell amplification efficiency was assessed on 30 individual lymphocytes. Amplification rates of the mutation and the marker were both 100% with an allele drop out rate of 0 and 15% respectively. The PGD procedure involved a standard IVF protocol, resulting in 2 embryos at day 3. The four blastomeres tested were mutation-free, and this result was confirmed by the marker analysis, showing the wild type allele. The two embryos were transferred, and a singleton pregnancy is ongoing. These results suggest that PGD is a valuable alternative to prenatal diagnosis for tuberous sclerosis.

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Development of an improved PGD test for Fragile X syndrome. *J. Steffann[1], N. Frydman[2], N. Gigarel[1], R. Gesny[1], J-P. Bonnefont[1], G. Tachdjian[2], V. Kerbrat[3], E. Feyereisen[3], R. Frydman[3], A. Munnich[1], P. Burlet[1].* 1) Service de génétique, U393, Hôpital Necker-Enfants Malades, Paris, France; 2) Biologie de la reproduction, Hôpital Antoine-Béclère, Clamart, France; 3) Service de gynécologie-obstétrique, Hôpital Antoine-Béclère, Clamart, France.

Fragile X (FX) syndrome is the most common familial cause of severe mental retardation inherited as an X-linked trait caused by expansion of a CGG repeat in the FMR1 gene. Both males and females can be affected leading to a high recurrence risk for this disorder, so that at-risk couples often ask for preimplantation genetic diagnosis (PGD). PGD involves removal of single cells from preimplantation embryos and analysis using highly sensitive PCR amplification. PGD of FX is difficult because of the large number of CGG repetitions in affected, precluding any attempt at sizing the mutant allele. Therefore two alternative methods are used for blastomere analysis: the detection of the non expanded allele, or the use of linked polymorphic markers. Direct detection of the normal alleles has been described but remained difficult due to the lack of PCR sensitivity. The main drawback of the indirect method is the non-informativity of the marker. Recently, multiple displacement amplification (MDA) has been reported to yield large quantities of high-quality DNA from single-cell. In order to increase the sensivity and the reliability of the FXS diagnosis, we developed a combined analysis of polymorphic markers with the study of the CGG normal allele, using MDA as a first step. Single-cell amplification efficiency was assessed on 56 lymphocytes for both normal CGG alleles and four linked microsatellite markers: FRAXAC1, DXS998, DXS1215, DXS548. Amplification and allele drop-out rates ranged from 88 to 95% and 4 to 34%, respectively. A couple requesting PGD was shown to be informative for both the expansion and the markers. A standard IVF protocol was performed resulting in 8 embryos including 4 shown to be unaffected. Two healthy embryos were transferred, resulting in a singleton pregnancy. PGD results were confirmed later in the four carrier embryos. In conclusion, MDA as a first step is a powerful tool for PGD in Fragile X.

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Expression Microarray Analysis to Detect Unique Fetal Markers and Fetal-Maternal RNA Trafficking in Maternal Whole Blood. *J.L. Maron[1], K.L. Johnson[1], C.Q. Lai[1], D. Slonim[2], D.W. Bianchi[1].* 1) Division of Genetics, Tufts Univ. School of Med., Boston, MA; 2) Department of Computer Science, Tufts Univ., Medford, MA.

Background: The rapid clearance of fetal nucleic acids from the maternal circulation following delivery presents an opportunity to identify unique, gender-independent markers. We used expression microarrays to study differences between peripartum women and their newborns. Methods: Paired sample sets were obtained from pregnant women immediately prior to term delivery and 24 hours postpartum, along with their newborns umbilical cord blood. Controls were non-pregnant women. Total RNA was extracted from whole blood samples in accordance with the PAXgene blood RNA kit protocol. Successful RNA extraction was confirmed using real-time RT-PCR for the housekeeping gene GAPDH. RNA was amplified in accordance with the Affymetrix One-step Amplification Protocol. Samples were hybridized to the Affymetrix HGU133a microarrays, scanned with the GeneArray Scanner and analyzed using the GeneChip Microarray Suite 5.0 (Affymetrix). Results: We successfully isolated, amplified and hybridized mRNA from maternal and newborn whole blood to oligonucleotide microarrays. Umbilical cord blood consistently had greater amounts of extracted and amplified mRNA. Comparative analysis between genes expressed in both the antepartum and the corresponding umbilical samples, but absent in both the postpartum and the control samples, identified a subset of genes that are presumably fetal in nature. Conclusions: Preliminary results suggest that comparison expression microarray analysis between a peripartum pregnant woman and her infants umbilical cord blood can be utilized to identify unique fetal markers, to monitor fetal-maternal RNA trafficking, and to potentially develop predictive models of pregnancy-related disease.

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Joubert syndrome: a paradigm for prenatal diagnosis of specific hindbrain malformations by ultrasound and MRI. *D. Doherty[1], D. Nyberg[2], J.R. Siebert[3], M.A. Parisi[1], D.W.W. Shaw[1], P.J. Strouse[3], P.F. Chance[1], M. Barr, Jr[3], B. Maria[4], I.A. Glass[1].* 1) University of Washington, Seattle, WA; 2) The Fetal and Women's Center of Arizona, Phoenix AZ; 3) University of Michigan, Ann Arbor, MI; 4) Medical University of South Carolina, Charleston, SC.

Malformations of the hindbrain (cerebellum, pons and medulla) affect approximately 1/5000 births (Metropolitan Atlanta Congenital Defects Program, personal communication). Although advances in brain imaging technology have facilitated identification of hindbrain malformations, difficulty in assigning precise diagnoses has created significant challenges for counseling patients and families regarding etiology, prognosis and recurrence risk. Disorders with cerebellar vermis hypoplasia (often labeled Dandy-Walker variant) represent a particularly challenging diagnostic group due to the many underlying causes and broad spectrum of outcomes. One of these disorders, Joubert syndrome (JS), is an autosomal recessive hindbrain malformation disorder characterized by cerebellar vermis hypoplasia, thick superior cerebellar peduncles and a deep interpeduncular fossa (the molar tooth sign (MTS)). Clinical signs include congenital hypotonia, ataxia, developmental delay, disordered respiratory control and abnormal eye movements. While the recurrence risk for JS is 25%, prenatal diagnosis using DNA testing is not clinically available for the vast majority of families, and the MTS has yet to be described using fetal MRI. We report our ultrasound and MRI results on fetuses with JS at 22-34 weeks, demonstrating that cerebellar vermis hypoplasia is present from early in gestation, and that the MTS can be identified well before birth. While we have not yet observed the MTS before 24 weeks gestation, we have accurately diagnosed JS before 22 weeks in one at-risk fetus and excluded JS in five other at-risk fetuses. In addition, we present correlation with fetal neuropathology and postnatal imaging. We propose an imaging strategy for the accurate in utero diagnosis of JS as an initial step toward differentiating between diverse hindbrain malformations to provide more accurate information regarding prognosis and medical management.

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Prenatal diagnosis of acute promyelocytic leukemia in a fetus at 28 weeks gestation. *E. Kolomietz[1], EJT. Winsor[1], G. Ryan[2,3], G. Seaward[2,3], S. Keating[1], C. Wang[1], D. Chitayat[2,4].* 1) Pathology/Laboratory Medicine, Mount Sinai Hosp/Univ Toronto, Toronto, Ontario, Canada; 2) Dept. of Ob&Gyn, Mount Sinai Hosp/Univ Toronto, Toronto, Ontario, Canada; 3) Fetal Medicine Unit, Mount Sinai Hosp/Univ Toronto, Toronto, Ontario, Canada; 4) Prenatal Diagnosis and Medical Genetics Program; Mount Sinai Hosp/Univ Toronto, Toronto, Ontario, Canada.

Fetal leukemia is an extremely rare disease and in almost all reported cases the fetus had Down syndrome. We report a fetus that presented with hydrops fetalis due to promyelocytic leukemia. The mother was a healthy 30 year-old G1P0. Ultrasound examination at 27 weeks gestation revealed hydrops fetalis including pleural effusions, ascites and skin edema. Attempts to karyotype the fetus by amniocentesis and cordocentesis failed since neither specimen produced metaphases in culture. Cytogenetic analysis was successful on pleural effusion and repeat amniotic fluid obtained at 28 weeks gestation. There were multiple single cell chromosome aberrations in both specimens. The most consistent aberration was an unbalanced derivative of chromosome 16 which resulted from a translocation between chromosomes 16 and 17 [der(16)t(16;17)(p13.3;q21)]. This was found in the amniotic fluid, but not in the pleural fluid. Spontaneous demise occurred at 33 weeks gestation. At delivery, the fetus was macerated with severe hydrops. The full blood count on a suboptimal specimen collected about 24 hours after birth revealed extreme leukocytosis with a white cell count 110 x 109 /L. The peripheral blood film showed immature granulocytes with features of promyelocytes. The morphological findings resembled those in acute promyelocytic leukemia. Molecular and FISH studies to check for involvement of the RAR-alpha gene on chromosome 17 are in progress.

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Prenatal diagnosis of dysmorphic syndromes. *C. Stoll, Y. Alembik, B. Dott, MP. Roth.* Genetique Medicale, Faculte de Medecine, Strasbourg, France.

Some dysmorphic syndromes can be detected prenatally(DPN) by ultrasound examination(US).The objectives of this study was to evaluate the prenatal diagnosis of dysmorphic syndromes by routine US. The material for this study came from our population based registry of congenital anomalies.The study period was 1992 to 2003. Results :we report the dysmorphic syndromes in categories of congenital anomalies. There were 564 cases with congenital heart diseases and 110 of them had recognized syndromes :86 chromosomal anomalies(23 % of them were diagnosed prenatally) and 24 non chromosomal syndromes,including 6 22q11microdeletions(3 DPN) ;4 situs inversus(3 DPN) ;3 VATER association(2 DPN) ;and 11 others :one each FAS,Ellis-Van Creveld,Greig,OFD, Chondrodysplasia punctata and Roberts detected prenatally and one each CHARGE,Aarskog, OAVS,Robinow,and SLO not detected prenatally.Theprenatal detection rate for the chromosomal syndromes and for the non chromosomal syndromes were 86 % and 80% for renal malformations,50% and 62% for limb reduction defects,71% and 83% for abdominal wall defects,80% and 100% for diaphragmatic hernia,37% and 43% for intestinal atresias,82% and 82% for cleft lip/palate,and 100% and 83% for neural tube defects respectively.Among the non chromosomal syndromes non detected were 3 VA(C)TER(L),3 osteochondrodysplasias,3 FAS,and one each amniotic band, Hanhart,Fanconi,Aarskog,De Lange,Di George,TAR,osteogenesis imperfecta, Goldenhar,OFD,Pierre Robin,Meckel-Gruber,caudal regression,OEIS,and Fryns. Detection rate varies with the type of syndrome and increases with increasing numbers of abnormalities in the fetus.For all categories of congenital anomalies apart from renal ones,the detection rate is much higher for associated anomalies than it is for isolated anomalies. However,prenatal ultrasound screening allows diagnosis of a large proportion of chromosomal syndromes and recognized dysmorphic syndromes without chromosomal anomalies.

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Perinatal outcome in women with combined neural tube defects and Down syndrome screening positivities. *S. Kim, S. Bai.* OB/GYN, Col Med, Seodaemun Ku, Yonsei Univ, Seoul, Korea.

To evaluate the perinatal outcome of patients with serum screen-positive for both neural tube defects(NTD) and Down syndrome(DS). Alpha-fetoprotein(AFP),unconjugated estriol(uE3) and intact human chorionic gonadotropin(hCG) levels were determined with RIA kits at 15-20 weeks of pregnancy.Estimates of individual DS risk were made using the concentrations of these analytes and maternal age.We have selected cases with both an AFP value 2.5 multiples of median(MoM) and a DS risk more than 1:270 at second trimester (dual positivity). We have analyzed all data about the perinatal outcomes.Dual positivity was found in six cases.There were one anencephaly and 1 open spina bifida.Karyotypes were not performed and pregnancies were terminated.In four remaining cases the fetal karyotypes was normal.Among these 4 cases,one showed severe preeclampsia associated with fetal growth restriction and delivered at 29 weeks.In the other 3 cases,the perinatal outcomes was uneventful.Our findings suggest that patients with dual positivity at NTD/DS serum screening may have an increased risk of adverse perinatal outcome.

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Maternal steroids and the non-invasive diagnosis of low-estriol disorders. *J. Marcos[1], W.Y. Craig[2], C. Shackleton[1].*
1) Children's Hospital Oakland, Oakland, CA; 2) Foundation for Blood Research, Scarborough, ME.

Introduction: Unconjugated estriol (uE3) is measured during second trimester prenatal screening. While estriol itself has little physiological importance, low values can indicate the presence of a disorder in feto-placental synthesis with potential health implications for the baby. Since fetal steroids pass through the maternal circulation and into the urine, analysis of maternal fluids can be used for diagnosing individual causes of low estriol. **Methods**: Samples of urine and serum were obtained from >1,000 pregnant women who exhibited low uE3 (<0.3 MoM), and most were collected as part of a multicenter study to evaluate potential screening for Smith-Lemli-Opitz syndrome (SLOS). The sample (1ml) was hydrolyzed, extracted, derivatized and analyzed by GC/MS for compounds diagnostic of SLOS, STS and other disorders. Key analytes for SLOS detection were dehydropregnanetriols and dehydroestriol, and the key parameter for STS was excessive excretion of 16-hydroxy-DHEA sulfate. **Results**: Quantitative ratios between diagnostic steroids (*precursor metabolite/product metabolite*) have been evaluated. Cut-off values for these in urine and serum were established to optimize the sensitivity and specificity. The false positive rate for SLOS was 0%, and for STS was 3.2%. The false negative rate for both SLOS and STS was 0%. From these selected ratios 5 pregnancies positive for SLOS, and 182 positive for STS were detected. Confirmatory diagnostic data were available through independent testing and/or postnatal clinical examination for all SLOS cases and 50% of STS cases. Independent of above study, we also present data on another low-estriol condition, fetal P450 oxidoreductase deficiency (ORD).**Conclusions**: For STS and SLOS, non-invasive second trimester diagnosis is readily attainable through GC/MS analysis. In contrast to FISH testing, the method detects STS caused by point mutations. Analytes have also been included in the panel that should allow detection of OR, 17-hydroxylase and aromatase deficiencies.**Acknowledgements**: Funding was from the NIH (R01 HD38940). The contribution of multiple investigators in participating institutions is acknowledged.

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PLACENTAL mRNA IN MATERNAL PLASMA AND ITS CLINICAL APPLICATION TO THE EVALUATION OF PLACENTAL STATUS IN A PREGNANT WOMEN WITH PLACENTA PREVIA-PERCRETA. *S. Miura, H. Masuzaki, K. Miura, T. Ishimaru.* Dept OB/GYN, Nagasaki Univ Biomed Sci, Nagasaki, Japan.

Objective: We applied placental mRNA measurement in maternal plasma to the evaluation of residual placenta condition in a pregnant woman with placenta previa-percreta (PPP) and bladder invasion. Material and Methods: Although a supra-vaginal hysterectomy just after the cesarean section (the first surgery) was done at 37 weeks of gestation, a 16-cm placental mass close to the internal os of the uterus could not be removed. Therefore, therapy with methotrexate (MTX) was initiated four times after the first surgery to aid resorption of the placental residue, and then a second surgery was performed to complete the hysterectomy. Blood samples were collected at intervals, and before/after surgeries. Human chorionic gonadotropin (hCG) and human placental lactogen (hPL) were selected as representative placental mRNAs, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured as a housekeeping gene. A one-step real-time RT-PCR assay was performed to measure the concentration of mRNA in maternal plasma. Result: The hCG mRNA showed a decreasing tendency similar to the pattern for hCG protein levels measured by an immunoradiometric assay , but exhibited a transient increase after the first MTX therapy and then decreased again to <10 copies/ml after the second surgery. Interestingly, plasma hPL mRNA levels showed a similar pattern but rose again after the second MTX therapy, while the hCG mRNA level decreased. The GAPDH mRNA concentrations in plasma also increased after both surgeries and after the first MTX therapy. Conclusion: Real-time quantitative RT-PCR adopted in the present study is a sensitive method to monitor changing mRNA levels resulting from apoptotic effects in the placenta, and to evaluate invading conditions of the trophoblastic villus.

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Uncommon Mutations Have Been Found During Prenatal Diagnosis of More Than 1200 Iranian Families Using DNA Sequencing. *B. Azimifar[1, 3], M. Eram[1], M. Masrouri[1], V. Lotfi[1], P. Foulady[1], M. Hosseini[1], A. Abdolhosseini[1], S. Zeinali[1, 2].* 1) Medical Genetics Lab of Dr. Zeinali, Tehran, Iran; 2) Department of Biotechnology, Pasteur Institute, Tehran, Iran; 3) Department of Biology, Shahid Beheshti University, Tehran, Iran.

Beta thalassemia is the most common hereditary severe blood disorder in Iran. Prenatal diagnostic (PND) strategies for Î²-thalassemia by now are one of the most effective means for preventing the birth of affected children. More than 1117 prenatal diagnosis had been completed by our lab since mid 2000. Besides routine ARMS-PCR methods were used for screening the most common Iranian Î²-globin gene mutations, more rigorous molecular analyses were performed for the 58 unresolved cases (5% or 58/1117) using DNA sequencing. The total of 22 rare Î²-globin gene mutations has been found by us yet. There were 4 new mutations [i.e. codon 37/39 (-GACCCAG), codon 37 (GA), codon 44 (-C) and IVSII nt 850 (GC)] which had not been reported in Iran, previously. The knowledge of mutation types, their frequencies and hematological data of the carrier individuals could be useful for developing Î²-thalassemia molecular screening plan in Iran and other neighbor's countries.

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Decrease in mitochondrial cytochrome c oxidase III and ATPase 6 levels and mutations in the promoter region in preeclamptic pregnancies. *D. Cha, S.H. Lee, C.N. Lee, K.J. Lee, S.W. Cho, J.B. Chang.* Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea.

Objectives: The placenta transports nutrients and oxygen to the fetus. In active transport, energy is expended to transport material against a gradient. Preeclamptic pregnancies are often associated with an undernourished fetus, suggestive of a reduction in placental transport. To clarify the possible relation between mitochondrial gene expression and the placental dysfuction in preeclampsia, we examined the expression of cytochrome c oxidase III and ATPase 6. The promotor region was also investigated to detect any mutation in H-strand and L-strand. Materials and Methods: Placental tissues were obtained from preeclampsia and control group(each 5 cases) between 22 and 41 gestational weeks. The expression of cytochrome c oxidase III and ATPase 6 were analyzed by RT-PCR and quantified by the densitometry. The H-strand and L-strand of promotor region were analyzed by ABI prism 377 DNA analyzer after PCR. Results: The expression of cytochrome c oxidase III and ATPase 6 levels were reduced in the preeclampsia than in the control group. The mutation of L-strand promotor region was not detected in both group. In H-strand, some mutations including T insertions(553, 557, and 560), substitution(C567T), and mutated AAA(563-565). Conclusions: The decreased level of the mitochondrial gene(cytochrome c oxidase III and ATPase 6) is involved in placental dysfunction in preeclampsia. The reduced expression of these genes suggests malfunctioned transcription in the promotor region. The verified mutations in the promotor region may support this suggestion.

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Evaluation of the fetus with multiple skeletal anomalies. *E. Severin, D.F. Albu, C. Albu.* Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania.

Background.Skeletal anomalies are bone and cartilage disorders and most of these are genetic conditons.With early detection, antenatal screening, patients can avoid or minimize the complications associated with this disorders.Objective.To specify the role of 3D ultrasonography as a screening tool for prenatal identification of specific skeletal anomalies in skeletal dysplasias. Patients and Methods. A 34-year-old Caucasian female was referred at 19 weeks' gestation for a routine prenatal ultrasound. The patient was examined with Color Doppler and 3D system.We also collected information about family medical history. Amniotic fluid samples were taken to perform prenatal molecular diagnosis.Parental studies were made for recurrence risk counseling. Results. The patient's obstetrics history revealed three previous miscarriages, all in the second trimester (unknown causes). Her third pregnancy ended at 24-week with stillborn fetus (thanatophoric dysplasia morphology appearance but no radiologic examination or molecular confirmation were made). A 3D ultrasound examination of fourth pregnancy identified a single fetus with skeletal findings suggestive of thanatophoric dysplasia type II: micromelia with straight femurs and cloverleaf skull deformity. More associated anomalies were detected.Clinical suspicion was confirmed by molecular diagnosis. The couple chose to terminate the pregnancy. Conclusions.Multiple skeletal anomalies were detected by ultrasound in the prenatal period. The pattern of skeletal anomalies suggested thanatophoric dysplasia. Molecular confirmation was possible. Evaluation and genetic counseling based on both 3D ultrasonography and molecular genetic testing should be considered for the next pregnancy.

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Prenatal diagnosis of a fetus with lip-jaw-palatal cleft. *D.F. Albu, E. Severin, C. Albu.* Human Genetics, Carol Davila Univ. Med and Pharm, Bucharest, Romania.

BackgroundVisualization of the fetal face has acquired a special importance because noticeable features in the phenotype facilitate the diagnosis of syndrome-like or chromosomally induced clinical conditions. Prenatal screening offer the possibility of preventing the birth of infants with serious congenital abnormalities. **Objective**. To specify the most appropiate safety method of trisomy 13 prenatal screening. **Patient**. A 27-year-old Caucasian female, pregnant for the second time, underwent to routine ultrasound examination at 16 weeks of pregnancy. The woman and her husband had normal general health and were not genetically related. There was no history of abnormal children in their families. **Methods**: routine ultrasonography at 16 weeks of pregnancy; selective ultrasonography for the detection of fetal abnormalities; triple test; amniocentesis(amniotic fluid samples were taken at 17 weeks); chromosome analysis in cultured fetal cells was performed following the standard protocols. **Results**. Ultrasound examination revealed a single prenancy with an abnormal foetal facial development: lip-jaw-palatal cleft. This sonographic marker indicated the possibility of a chromosomal anomaly, such as trisomy 13 or trisomy 18. Lip-jaw-palatal cleft is typical, but not specific for trisomy 13. Triple test was not sensitive to the presence of trisomy 13. Chromosome analysis was recommended and fetal karyotype demonstrated 47,xy,+13. After an extensive counseling the parents decided to terminate the pregnancy with trisomy 13. **Conclusions**. In this case report, the lip-jaw-palatal cleft was a strong and useful screening tool for the prenatal detection of trisomy; chromosomal analysis is recommended in all cases with similar types of facial cleft diagnosed prenatally because many of the features of trisomy 13 and trisomy 18 overlap.

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Perinatal and neurodevelopmental outcome with isolated fetal ventriculomegaly: A systematic review. *M.D. Laskin[1], J. Kingdom[1], A. Toi[2], D. Chitayat[3], A. Ohlsson[4].* 1) Department of Obstetrics and Gynecology , Mount Sinai Hospital; 2) Department of Medical Imaging, Mount Sinai Hospital; 3) The Prenaal Diagnosis and Medical Genetics Program, Mount Sinai Hospital; 4) Department of Pediatrics, Mount Sinai Hospital, Toronto, Ontario, Canada.

Objective: To establish the perinatal and neurodevelopmental outcomes of fetuses diagnosed with isolated ventriculomegaly (IVM). Methods: A systematic review of cohort, case/control studies, case series and case reports of IVM [unilateral or bilateral enlargement of the lateral ventricle (>10 mm) with no additional diagnosis at the time of the initial ultrasound], identified by searching without language restrictions The Cochrane Library, MEDLINE and Embase databases in June 2004. Results: No cohort study was retrieved but 518 cases were identified in one case/control study and 29 case series/case reports. Of 206 cases with follow-up and known gestational age at diagnosis, normal developmental outcomes were found in 82%, mild impairments in 9% and moderate/severe impairments in 10%. Prenatal diagnosis could have reduced the risk of moderate/severe impairments to 7%. In 83 cases followed to at least 20 months of age, 74 % had normal neurodevelopment, 11% were mildly delayed and 16% had moderate/severe developmental delays. Conclusions: Outcome was favorable in 82% of IVM cases, decreasing to 74% when cases are followed to at least 20 months of age. We recommend prospective cohort studies with new diagnostic modalities to better identify the underlying conditions relating to moderate/severe impairments in the remaining cases.

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Prenatal Diagnosis of Steatocystoma Multiplex of the scalp using fetal ultrasound and MRI. *R. Teitelbaum[1], S. Blazer[4], A. Toi[3], K. Fong[3], N. Okun[2], D. Chitayat[1,2].* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto ON, Canada; 2) Department of Obstetrics and Gynecology, MSH, Toronto ON, Canada; 3) Department of Medical Imaging, MSH, Toronto ON, Canada; 4) Division of Neuroradiology, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto ON, Canada.

Steatocystoma multiplex (OMIM184500) is an AD condition associated with benign round/oval dermal cysts widely distributed on the body. It is caused by a mutation in the keratin 17 gene(KRT17). We report the diagnosis of these scalp lesions, which can resemble encephalocele, in two fetuses using ultrasound and MRI. (1) The couple was healthy, non-consanguineous and of Caucasian descent. The 31yr-old G1P0 mother reported a history of two subcutaneous lesions on her scalp. Her father and identical twin sister also had subcutaneous cysts. In this pregnancy detailed fetal ultrasound revealed a 2x1.5cm cystic lesion protruding from the right frontal bone. The cystic lesion appeared to be separate from the bone and had no solid elements. A fetal MRI utilizing the HASTE sequence at 22weeks gestation revealed a 1.6x0.8x2.1cm midline encapsulated cystic structure within the scalp without continuity with the intracranial structures. The findings were consistent with a subcutaneous cyst. A male child was delivered at term by an uncomplicated SVD. On initial examination, a subcutaneous scalp cyst was palpated and regressed substantially since birth. His growth and development are normal. (2) The parents were healthy and non-consanguineous and of Caucasian descent. In this pregnancy, amniocentesis showed a normal female karyotype. A detailed fetal ultrasound at 19 weeks gestation showed a cystic mass overlying the left frontal bone near the coronal suture raising the possibility of an encephalocele. No distinct connection has been demonstrated to the subarachnoid space. The underlying brain and the rest of the fetal anatomy appeared normal. Fetal MRI showed a well defined T2 hyperintense 7mm lesion in the left scalp. There was no intracranial connection and no cranial abnormalities were detected. The findings were consistent with a dermoid cyst.

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Renal Tubular Dysgenesis (RTD. Report of Three Cases. *M. Thomas.* Dept Medical Genetics, Mount Sinai Hosp, Toronto, ON, Canada.

Renal Tubular Dysgenesis (RTD). Report of Three Cases. M. Thomas1, S. Keating2, A. Toi3, G. Ryan4, D. Chitayat1 Mount Sinai Hospital, Dep. Of Obstet&Gyn, 1) The Prenatal Diagnosis and Medical Genetics Program, 2)Fetal Medicine Unit, 3)Dept. of Pathology and 4) Dept. of Diagnostic Imaging, University of Toronto, Toronto, Ontario, Canada. RTD is a lethal autosomal recessive condition caused by a defect in differentiation of the proximal and distal convoluted tubules. It is associated with oligohydramnios in later pregnancy. Most cases are associated with calvarial hypoplasia, which may provide a tool for early prenatal diagnosis. Case 1: The parents were of Sri Lankan descent and non-consanguineous. Their pregnancy was uncomplicated until 22.6 weeks gestation when fetal U/S showed enlarged and echogenic kidneys. U/S at 26.7GA showed 24W size fetus with anhydramnios. The pregnancy was terminated and autopsy showed RTD, Potter sequence, and hypoplastic calvarium with prominent sutures. Case 2: The parents were of French-Canadian origin and non-consanguineous. A fetal U/S at 19W gestation was normal. At 24GA the U/S showed severe oligohydramnios with normal-looking kidneys. The pregnancy was terminated and the autopsy showed RTD, collapsed hypoplastic calvarium with prominent fontanels Case 3: A sib of case 2. The pregnancy was uncomplicated until 23 weeks gestation when the U/S showed severe oligohydramnios. The pregnancy was terminated and the autopsy showed RTD, hypoplastic calvarium with prominent fontanels and sutures. The possibility of RTD should be raised in late onset oligohydramnios. The finding of calvarial hypoplasia and large fontanels can help in making the diagnosis and in earlier PND in couples at risk.

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The prenatal diagnosis of femoral facial syndrome. *A.F. Wagner[1], L.M. Hill[2], E. McPherson[3], W.A. Hogge[1].* 1) Dept. of OB/GYN/RS, Div. of Reprod. Genetics, Magee-Womens Hosp., Pittsburgh, PA; 2) Dept. of OB/GYN/RS, Div. of Ultrasound, Magee-Womens Hosp., Pittsburgh, PA; 3) Medical Genetics, Marshfield Clinic, Marshfield, WI.

We report a case of a prenatally-diagnosed skeletal dysplasia. A 39yo G_5P_4 was referred for a targeted ultrasound at 26.9wks after an outside scan demonstrated short bowed femurs, micrognathia and clubbed feet. She had insulin-requiring diabetes. Amniocentesis showed a 46,XX karyotype. The long bones except the humerus were short. Both femurs were short and bowed(19.4wks). Micrognathia and the clubbed feet were confirmed. At 32.3wks, the femurs were consistent with 22.7wks. All other long bones except the humerus were shortened (27-29wks). A small right pelvic kidney was seen. The diagnoses of femoral facial syndrome and bilateral proximal femoral focal deficiency were entertained. At 35.9wks, the femur and tibia now measured 10 and 6 weeks behind. Due to placenta previa, she had a C/S at 37.1wks. The weight and HC were within normal limits. There was right microtia with abnormal folding of cartilage on the left. She failed the hearing screen. There was a cleft palate consistent with Pierre-Robin sequence. Thumbs were digitalized. Lower arms were shorter than the upper. There was excess skin at the elbows. Feet were clubbed bilaterally. Femurs were extremely short. Hips were fixed and externally rotated with dimpling. Her skin along the femurs and groin was smooth without any creases. Knees could flex but not extend past 40. On renal U/S, the right kidney was small. An echocardiogram and head U/S were normal. Neonatal evaluation considered femoral facial and Roberts syndrome. Peripheral blood karyotype was 46,XX with no evidence of premature centromere separation. Femoral facial syndrome was diagnosed. She was discharged to a step-down unit from the NICU and then home. She has been evaluated by plastic surgery and speech therapy and had a heel cord lengthening. This case demonstrates the successful prenatal diagnosis of femoral facial syndrome. The appearance of femoral hypoplasia with micrognathia, cleft palate, malformed ears, and maternal diabetes is consistent with this diagnosis.

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Power to detect genotype × environment interaction in continuous environments. *V.P. Diego, T.D. Dyer, L. Almasy, J.W. MacCluer, J. Blangero.* Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

There is growing interest in the possible role of genotype × environment interaction in human variation. We carried out power analyses of genotype × environment interaction along a continuous environment by analytically deriving the Fisher information (FI) matrix under our model and numerically computing this FI matrix for a specific data set, namely the San Antonio Family Heart Study (SAFHS), at a fixed sample size, N = 690. In general, the null hypothesis of no genotype × environment interaction corresponds to two hypotheses on the genetic effects across the environmental continuum: 1) the additive genetic variance is homoscedastic, and 2) the genetic correlation is not different from 1. Under our model, heteroscedasticity in the additive genetic variance is indicated by a slope parameter in the additive genetic variance that is significantly different from 0. Similarly, a genetic correlation different from 1 is indicated by a slope parameter in the genetic correlation that is significantly different from 0. As an example, we examined an empirical case of genotype × age interaction using data on the insulin-like growth factor I (IGF-I) axis in Mexican American families participating in the SAFHS. We found significant genotype × age interaction for IGF-I due to heteroscedasticity in the additive genetic variance and to a genetic correlation different from 1. Given the observed parameter estimates, we computed the FI matrix, the Wald statistic and power for a range of parameters. We found that the Wald statistic is unbiased and the power functions are monotonic, thus indicating a well-behaved test statistic. The power to detect the slope parameter in the additive genetic variance at the observed effect size was 0.9976 and the power to detect the slope parameter at the observed effect size in the genetic correlation was 0.9334. Thus, there was adequate power to detect genotype × age interaction for IGF-I in our study.

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An admixture model to identify genotyping errors via Hardy Weinberg disequilibrium. *H. Seltman.* Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA.

Detection of markers for which errors in the determination of allele status are systematically present is an important pre-processing step in linkage and association analysis. Problematic markers often lead to an excess of homozygotes in the sample genotypes and hence are typically identified by conducting tests for Hardy Weinberg (HW) equilibrium. This approach is complicated by the presence of population substructure. With large samples even minor substructure and misclassification of ancestry of subjects can lead to numerous violations of HW. To circumvent these difficulties we develop a Bayesian hierarchical model that simultaneously models recent populations as a mixture of ancient continental populations and models population/marker HW disequilibrium as deviation from the HW homozygosity rate. The output of this model is a matrix of estimates of disequilibrium by recent population group and by marker that can be used for exploratory data analysis to detect problematic markers that have high disequilibrium or markedly different levels of disequilibrium across populations.

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PANCPRO: a Mendelian Risk Prediction Model for Pancreatic Cancer. *W. Wang[1], A.P. Klein[2,3,4], S. Chen[1,5], G. Parmigiani[1,2,3].* 1) Biostatistics, Johns Hopkins School of Public Health; 2) Oncology, Johns Hopkins School of Medicine; 3) Pathology, Johns Hopkins School of Medicine; 4) Epidemiology, Johns Hopkins School of Public Health; 5) Environmetal Health Science, Johns Hopkins School of Public Health.

Pancreatic cancer is the 4th leading cause of cancer death in the United States. Given that the 5-year survival rate is 5% and surgical resection of early lesions offers the best chance for survival, early detection is critical to reducing the disease burden. Numerous studies demonstrate that individuals with a family history of pancreatic cancer are at an increased risk. While segregation analysis showed evidence of genetic effects, the genetic basis of the majority of this familial aggregation has yet to be identified. However, even in the absence of precise genetic testing, identification of individuals who are at high risk of developing pancreatic cancer and who may benefit from screening is of great importance. To this end we developed PANCPRO, a Mendelian risk prediction tool for pancreatic cancer. Mendelian models use estimates of genetic effects (gene frequency, penetrance etc) and apply well-established Bayesian prediction techniques to obtain carrier probabilities. PANCPRO is built upon MARGENE, a C routine implemented in BayesMendel(available in R), a generic tool for building Mendelian risk prediction models. The underlying model for PANCPRO is based on the segregation model by Klein et al. 2002. PANCPRO gives the probability an individual carries a mutation at the major locus and the probability the individual will develop pancreatic cancer during a particular age interval. To validate this model we compared the predicted incidence of pancreatic cancer in about 900 families enrolled in the National Familial Pancreas Tumor Registry, to the actual number of prospective pancreatic cancers in the registry. Results indicate no significant difference between the observed and predicated number of cases, supporting the utility of this model. In conclusion, PANCPRO is a powerful tool for health care providers to help identify individuals who may carry a major gene that increases risk of pancreatic cancer.

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Twin Study Heritability Estimates of Central Auditory Processing Functions. *D. Drayna[1], C. Brewer[1], H. Snieder[2], C. Zalewski[1], T. Friedman[1], R. Morell[1].* 1) NIDCD/NIH, Rockville, MD; 2) Georgia Prevention Institute, Augusta, GA.

Sound is transduced by the cochlea to nerve impulses that are processed in the central auditory pathway. Skills and behaviors that comprise auditory processing include sound localization and lateralization, auditory performance in the presence of competing and/or degraded signals, and temporal resolution, masking, integration and ordering. Auditory processing disorders (APD) can arise due to central nervous system (CNS) disease and trauma, age-related changes in the CNS, and peripheral hearing loss. In children, APD is often comorbid with disorders that can be heritable, including dyslexia, stuttering, autism, and specific language impairment. The extent to which APD may contribute to, or share etiology with, these heritable disorders is controversial, and little is known about the heritability of auditory processing abilities in the general population. We administered several tests of auditory processing to healthy, same-sex identical and fraternal twins. We found variation in test performance, and significant differences between MZ and DZ correlations for performance on three tests of dichotic listening. In these tests, two different stimuli are presented simultaneously, one to each ear, and subjects are asked to identify both stimuli. While the stimuli differed in the three tests, the dichotic presentation was the same. We estimate heritability to be 0.74 (0.63-0.81, 95% CI), 0.71 (0.53-0.82) and 0.43 (0.16-0.64) for performance on these three tests, using multiple regression analyses within a Generalized Estimating Equation (GEE). These results that genetic approaches may be useful for understanding Auditory Processing Disorders.

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Estimation of the fraction of Usher syndrome families due to unidentified genes. *W.J. Kimberling, D. Orten.* Dept Genetics, Boys Town Natl Research Hosp, Omaha, NE.

Usher syndrome is the most common form of combined hearing and vision loss. There are 3 clinical types (1, 2, and 3) and 11 different genes responsible. Only 8 of these 11 genes have been identified. A modification of a novel method for the statistical analysis (Kimberling, in press, Hum Mut) of a genetically heterogeneous recessive disorder was used to estimate the fraction of different molecular subtypes of Usher syndrome as a function of the referring diagnosis. This methodology takes into account the fraction of cases remaining undiagnosed because no mutation was detected in any of the Usher genes. The analysis involved 304 Usher type I and 560 Usher type II families. Usher type II was excluded because the numbers were insufficient. For the Usher type I phenotype, the proportions were: 40% MYO7A, 17.7% CDH23, 10.6% PCDH15, 3.6% USH1C, with 3.3% due to the 4 other subtypes. By subtraction, 24.8% of the Usher I families were due to unidentified genes. For the Usher type II phenotype, the proportions are 78.4% USH2A, 7.3% MYO7A, 1.2% CDH23, 3.6% VLGR1, and 3.3% USH3. By subtraction, 6.2% of the families were due to unidentified genes. These results were then used to estimate the rates of false positive and negative molecular diagnoses conditioned on whether 0, 1, or 2 mutations were observed in one of the Usher genes. This data will have impact in the assessment of recurrence risk for genetic counseling of Usher syndrome families and on estimates of the reliability of the identification of cases for upcoming clinical trials. Further, the results show that a substantial number of Usher cases are due to as yet unidentified etiologies, which may or may not be genetic and recessive. So, in a related study, segregation analysis of families for whom no mutation has yet been observed showed no deviation away from that expected for an autosomal recessive trait. Thus, there is a compelling reason to believe that further investigation will reveal new recessively inherited genes involved in this disorder. This analysis also showed no evidence of digenic inheritance for Usher syndrome.

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Exclusion probability in a DNA mixture from a substructured population. *W. Niu, H.S. Lee, X. Sheng, R. Chakraborty.* Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Interpretation of data gathered for DNA-based human identification problems is also relevant for identifying contamination of foreign cells in samples of biological samples collected for tissue typing. DNA mixture, which provides signature of the presence of DNA from multiple sources, constitutes one such category of applications. Although likelihood-ratio based statistics provide relative supports of different alternative scenarios of mixture origin, when the mixture DNA profile is analyzed along with DNA profiles of suspected contributors, the concept of exclusion probability has also been used in this context. Probability of exclusion (PE) describes the chance of excluding a random individual (or cell type) as a part contributor of the mixed sample. Consequently, the PE computation uses data on the alleles present in the mixture sample alone, irrespective of the DNA profiles of the known subjects analyzed. In analogy of the PE computations in parentage analysis, the first suggestion of exclusion probability computations in DNA mixture analysis, used the assumption of Hardy Weinberg Equilibrium of genotype frequencies, which lead to the conclusion that the combined frequencies of all alleles in the mixture is the only determinant of PE. Later work in this area extended the computation of PE for substructured populations, again leading to the conclusion that the number of contributors in the DNA mixture does not affect the exclusion probability. With the use of the sampling theory of Dirichlet distribution of allele frequencies in a substructured population, we conclude that the exclusion probability is not independent of the number of contributors in the mixture. The chance that a random person would be excluded as a part contributor is in fact a composite function of the summed allele frequency, together with the presumed number of contributors, and the coefficient of coancestry in the substructured population. Numerical computations suggest that PE can be different depending on the number of contributors, even when the summed allele frequency remains the same. (Research supported by a grant from the Department of Development, State of Ohio).

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PEDMerge - A program for merging extended pedigrees: The CANHR Study. *Y. Wang, B.B. Boyer, A. Goropashnaya, G.V. Mohatt, R. Plaetke.* CANHR, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK.

At the Center for Alaska Native Health Research, we ascertain extended pedigrees for a genetic study of obesity and type 2 diabetes mellitus. In the field, we collect pedigree information using a pedigree drawing program. Pedigrees often become large. Therefore we often collect the information in sub-pedigrees that constitute one pedigree and finally need to be merged for data analyses. Merging these sub-pedigrees can be tedious and error prone even when using pedigree drawing programs. For example, in our study we have been repeatedly merging 26 sub-pedigrees to obtain one pedigree consisting of 986 individuals (recruited: 507) for genetic statistical analyses, since ascertainment is ongoing. In order to solve this problem, a Perl program PEDMerge - running across all platforms - was developed that merges multiple sub-pedigrees to one pedigree based on key individuals that are occurring in (multiple) sub-pedigrees and function as connecting points between sub-pedigrees. The size and number of sub-pedigrees to be merged are unlimited. Thus, each sub-pedigree may have multiple key individuals.

PEDMerge takes two input ASCII files: (1) pedin.txt, a standard pedigree file consisting of pedigree ID, ego ID, father ID, mother ID, sex and (2) keys.txt, a file that provides information about the key individuals specific IDs in each sub-pedigree. PEDMerge generates two output files: (1) pedout.txt, a file containing all merged pedigrees and unmerged pedigrees and (2) master.txt, a candidate Master file for the database PEDSYS developed by the Southwest Foundation for Biomedical Research (San Antonio, TX).

We will show (1) our approach to collect pedigree data in the field and (2) the application of PEDMerge.

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Genomic data mining to narrow down candidate disease genes for T1D. *X. Wang, Y. Xie, V. Magnuson, S. Ghosh.*
Max McGee Center for Juvenile Diabetes, Medical College Wisconsin, Milwaukee, WI.

Type 1 diabetes (T1D) is a complex genetic disease. It has become one of the most common chronic disorders for children. Identifying all the genetic factors contributing to the disease risk has been a challenge. We lack efficient means to identify all functional candidates beyond the HLA region and the insulin gene, as the exact disease etiology is still unclear; on the other hand genome scan studies offer too many positional candidates, with most of them bear no relationship to the disease. Here we describe a new approach that utilizes bioinformatics and data mining to narrow down the candidate genes. It identifies the genetic pathways that have enhanced representation among the positional candidates, and then identifies the key genes within these pathways that are also positional candidates. We apply it to a set of T1D genome scan data that we have, and the significance of the results is evaluated through Bootstrapping. Beside the expected findings such as pathways of antigen presentation, we found several new themes that are surprising, yet meaningful, including pathways highly relevant to normal beta-cell functioning, stress and cell death. These findings lead to protocols to prioritize positional candidates.

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Large scale analysis of gene-gene interactions. *W.S. Bush, S.M. Dudek, K.K Ryckman, M.D. Ritchie.* Center for Human Genetics, Vanderbilt University, Nashville, TN.

The development of high-throughput genotyping technologies has dramatically increased the number of polymorphisms considered in a typical genetic association study. These new technologies will dramatically change the state-of-the-art in studies of human disease. Detecting and characterizing interactions between these polymorphisms may be important in discovering genetic contributions to common complex diseases. Here, we present parallel multifactor dimensionality reduction (pMDR), a tool for extending the scale and scope of analyses of gene-gene and gene-environment interactions. MDR is a nonparametric, model-free approach developed for the detection of gene-gene and gene-environment interactions in the presence and absence of statistically detectable main effects. However, the previous implementation of MDR could not handle the computational scale of genome-wide association data. Thus, an extension utilizing the benefits of parallel computing was in order. To determine the power of pMDR to detect gene-gene interactions, we simulated 10 two-locus disease models with varying epistatic heritabilities (5% to 25%) and allele frequencies (.2/.8 and .4/.6). These models were used to simulate case-control datasets for each disease model with 50,000 SNPs. pMDR had 100% power to detect the disease loci for models with greater than 5% heritability. In the 5% heritability models, the disease loci typically appeared in at least one of the five cross-validation intervals. By assigning statistical significance with a post-analysis Bonferroni corrected chi-square statistic, only the disease loci were found significant. Each analysis was conducted on 24 pentium-4 processors of a computing cluster, and completed in 40 hours, demonstrating that the analysis of gene-gene and gene-environment interactions on this scale is computationally tractable with modest computing resources. This is a crucial step in facilitating our understanding of the genetic architecture of common, complex disease.

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Haplotype Inference for Tightly Linked SNPs in General Pedigrees. *K. Zhang[1], H. Zhao[2].* 1) Department of Biostatistics, Univ Alabama at Birmingham, Birmingham, AL; 2) Deparment Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

Haplotype reconstruction for tight linked markers in general pedigrees remains a challenging problem. Not only just a few methods are available to efficiently estimate haplotype frequencies and accurately infer haplotype configurations for general pedigrees, especially in the presence of missing data, but also performances of them have not been carefully evaluated. We have developed an efficient computer program, HAPLORE, for haplotype reconstruction and frequency estimation in general pedigrees with tightly linked SNP markers. Here, we compare and contrast HAPLORE with other two previously published methods (GENEHUNTER and PedPhase). We review the methods and point out the differences between them in terms of the models and computational strategies they use. Their performances are assessed through simulated haplotypes based on a real pedigree. Our results in the following table indicate HAPLORE outperforms the other methods. In the table, SAD is the sum of absolute difference between the true and estimated haplotype frequencies, IE (individual error) is the portion of individuals whose estimated haplotypes are not completely correct, and SE (similarity error) is the Hamming distance between the true and estimated haplotypes of an individual.

	Data Sets without Missing			Data Sets with Missing		
	HAPLORE	GENEHUNTER	PedPhase	HAPLORE	GENEHUNTER	PedPhase
SAD	0.0014	0.0401	0.0164	0.0297	0.1289	0.2953
IE	0.0015	0.0167	0.0079	0.0866	0.1054	0.1728
SE	0.0083	0.0801	0.0249	0.6236	0.5464	0.7248

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Open-source multifactor dimensionality reduction (MDR) software for detecting, characterizing, and interpreting gene-gene interactions. *N. Barney[1], W. Holden[1], B.C. White[1], L.W. Hahn[2], W. Bush[2], M.D. Ritchie[2], J.H. Moore[1].* 1) Computational Genetics Laboratory, Department of Genetics, Dartmouth Medical School, Lebanon, NH; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Multifactor dimensionality reduction (MDR) is a computational approach to detecting epistasis or gene-gene interactions. MDR has good power for detecting epistasis and has been successfully applied to genetic studies of amyloid polyneuropathy, atrial fibrillation, bladder cancer, breast cancer, coronary artery calcification, hypertension, myocardial infarction, prostate cancer, schizophrenia, and type II diabetes, for example. We describe here a comprehensive MDR software package with a user-friendly GUI that is programmed in JAVA and provided for free as open-source to the research community. Open-source MDR is distributed in three modules for 1) analysis, 2) permutation testing, and 3) data processing. The MDR analysis module carries out a combinatorial search for the best epistasis model as determined by n-fold cross-validation. Output includes statistics such as accuracy, sensitivity, specificity, odds ratios, and IF-THEN rules that facilitate interpretation. In addition to a text report of results, MDR provides publication quality graphical output of the distribution of cases and controls for each genotype combination. In addition, the fitness landscape of all evaluated models is provided in the form of line graphs and histograms that can be explored interactively through zoom and model identification features. The software can be run from a command line interface and will automatically run in parallel on multiprocessor computers with threading. The permutation testing module provides empirical p-values while the data processing module facilitates imputation of missing genotypes and resampling. Open-source MDR is platform independent and is freely available for download from Sourceforge.net which can be accessed from www.epistasis.org. Future features will include filter and wrapper methods to facilitate MDR analysis in genome-wide association studies.

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Kinship Estimation with an M-dependent Markov Model. *J. Ge[1], G. Zhang[2], R. Chakraborty[1,2].* 1) Dept Biomed Engineering, Univ Cincinnati, Cincinnati, OH; 2) Center for Genome Information, Dept. Environmental Health, Univ Cincinnati, Cincinnati, OH.

Kinship estimation, alternatively described as determination of biological relationships between individuals, is relevant in many research areas (e.g., biomedical sciences), forensic case-work, paternity testing, and victim identification in mass disaster cases. With genotype data from individuals, kinship can be estimated by using the maximum likelihood method. Assuming recombination between markers are independent (no genetic interference), identity-by-descent (IBD) states at an ordered map of markers represent a hidden Markov Chain. Although the precision of this model for estimating close relationships (e.g., full-sib) is satisfactory, its accuracy for second-degree relationships (e.g. avuncular) estimation is poor and unacceptable. We relax the assumption to allow genetic interference, and extend the simple Markov Model to OLE_LINK1an M-dependent Markov Model, in which IBD states of one marker are dependent on IBD states of multiple previous markers instead of only one previous marker. The computational complexity of this Model is large due to the need of calculating joint distributions of multiple IBD states for different kinships. Symbolic computing is a way to reduce the complexity. In addition, genotyping errors can reduce the precision of likelihood estimation, and population substructure increases the proportion of homozygous loci in individual's genome. These two factors are also incorporated to determine their effects on kinship estimation. Better performance of the M-dependent Markov Model has been achieved compared to a simple Markov Model for real datasets. This new model is also applied to simulation data to evaluate its performance with genotyping error and population substructure. (Research supported by a grant from the Department of Development, State of Ohio).

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A Novel Statistic for Testing Association of Pathway with Disease. *Z. Yu[1], W. Wang[2], L. Jin[3], M. Xiong[4].* 1) Probability and Statistics, Beijing, China; 2) Chinese National Human Genome Center at Shanghai; 3) School of Life Science, Fudan University, Shanghai; 4) Human Genetics Center, University of Texas Health Scince Center at Houston.

The new concept concerning complex disease is to assume that development of disease should be considered as a dynamic process with gene-gene and gene-environment interaction within a complex biological system, which is hierarchically, organized into complex gene interaction networks. . Traditional analytical methods for investigating the contribution of genetic variation to disease risk focus on the locus-by-locus paradigm, studying one locus, one haplotype, or one gene at a time. The locus-by-locus approaches are inadequate and inappropriate for addressing questions of the relationship between biological networks and phenotypes. Although the variation each gene contributes to disease risk is unidentifiable, the contribution of the set of genes in the network as a whole may be significant. Therefore, association of the biological networks with the disease will play an important role not only for understanding the mechanism of the diseases, but also for the development of efficient drugs. Study design and statistical methods for testing association of biological networks with disease will be difficult, but essential. New non-traditional analytical methods for testing association of biological networks with the disease and the biological interpretation of test results are desperately required. In this report, we will develop a general framework and statistics for testing association of biological networks with the disease The distribution of the statistics will be studied by simulation and analytic methods. The relationship between the association of biological networks and gene-gene interactions within and between pathways will be investigated. The proposed methods will be applied to atherosclerosis study to test association of inflammatory and antioxidant pathways with the disease.

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Effectiveness of maximization algorithm of area under the ROC curve for partitioning quantitative phenotypic value into genotypic value and environmental value. *S. Kamitsuji[1], N. Kamatani[2].* 1) JBIRC, JBiC, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Algorithm for partitioning quantitative phenotypic value into genotypic value and environmental value is proposed. An aim of Quantitative Trait Locus (QTL) analysis is to find a locus or haplotype contributed to an observed quantitative phenotye. Quantitative phenotype is the result of genetic factors, environmental factors, and the environmental factors that interact with the genetic factors, and it is natural to consider that the sum of these factors contributes to a variation in a population. For finding such a locus or haplotype, it is important to extract only genetic variation from the observed quantitative phenotypic value. Linear model, one of traditional statistical models, is frequently used for this implementation. However, it may be complicated to understand the result of linear model since estimated coefficients change by selecting coding method for genotype data. Such a burden is resolved by an introduction of notion of area under the Receiver Operating Characteristic (ROC) curve. ROC curve is a plot to show the trade-off between sensitivity and specificity. Accuracy of diagnostic test can be evaluated by the area under the ROC curve (AUC), which takes the value from 0 to 1. If diagnostic test has high accuracy, then AUC is closed to 1. In our study, it is assumed that genotypic value can be represented as the difference between quantitative phenotypic value and the value of linear combination of environmental factors. Under this assumption, the coefficients in linear combination above are estimated so as to maximize the AUC when the quantitative phenotypic value is divided into two parts in terms of genotypes on a target locus, and the modified quantitative phenotypic value can be calculated as subtracting the sum of product between each estimator and environmental value from the original value. The advantages of using the modified value over the original value or the value modified by a linear model are shown by giving a numerical example of predicting the haplotype contributed to quantitative phenotypic value using the computer algorithm QTLhaplo.

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Haplotype Tag SNPs Selection Using Support Vector Machines. *J. Li.* Computer Science, Case Western Reserve Univ, Cleveland, OH.

Tag SNPs selection is an extremely important problem for whole genome association studies with two important implications. First, genotyping efforts can be greatly reduced by only typing the tag SNPs for large scale whole genome association studies. Second, by removing redundant SNPs, statistical power of association analyses can be improved since the number of tests, which is usually proportional to the total number of SNPs, will be greatly reduced. Although the tag SNPs selection problem has been investigated intensively recently, challenges are remain. Most methods select the minimum SNPs that could capture the haplotype diversity. Other methods assess the predictive power of a SNP (or a set of SNPs) to other SNPs. But none of the above formulations have directly addressed the discriminative power of the subset of tag SNPs with respect to phenotypes. We argue that, for whole genome association studies of complex diseases, the redundancy of all the SNPs should be evaluated based on their relevance to the disease of interest. And we formulate the SNP selection problem as the feature selection problem under the supervised learning framework. More specifically, we employ a state-of-the-art learning algorithm called support vector machine (SVM) to rank SNPs. We take the case-control study design and assume that haplotypes of each individual are known. Polynomial kernels based on Hamming similarity of haplotypes are used to train the data. SNPs are ranked based on their contributions to the discriminate function. We evaluate the approach on simulated data as well as real datasets. The results show that for each dataset, a compact subset of SNPs with high discriminative power was retained for further association analysis.

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Complex Segregation Analyses of Bipolar Affective Disorder: Evidence for a Single Major Predisposing Locus.

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Background: The etiology of bipolar affective disorder (BP) is to still unknown. Genetic factors are known to contribute to the etiology of BP. Family studies have shown that the lifetime prevalence of BP in families of BP probands is higher that in families of psychiatrically healthy controls. We aimed to investigate the most likely mode of inheritance in a large sample of BP patients seen at the psychiatric clinic of a large university hospital. Subject and Method: Complex analysis segregation was carried out using 656 individuals distributed within 92 nuclear families from a sample of 99 unrelated BP probands. Each family member was seen by a single psychiatrist and interview using the Mini International Neuropsychiatric Interview Plus. Segregation analysis was conducted using the unified mixed model implemented in the computer program POINTER. Results and conclusion: Compared to the mixed Mendelian model the sporadic model was rejected (4GL, $p < 0.000$). The hypothesis of no major effect also was rejected (3GL., $p < 0.000$); whereas the hypothesis of no multifactorial component was not rejected (1GL., $p = 0.824$). The results indicate the presence of a major gene that influences BP. However, when recessive, dominant and additive models were compared with multifactorial transmission, none was accepted. The dominant mode was proved to be more suitable according to Akaike criterion (AIC = 527,816). In conclusion, our data supports the existence of a major predisposing gene to PB in our large sample of patients making it suitable for linkage and/or association studies.

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Mapping Tumor Suppressor Genes using Multipoint Statistics from Copy-Number Variation Data. *I. Ionita, B. Mishra.* NYU, New York, NY.

Microarray-based comparative genomic hybridization (array-CGH) approach now provides rapid genome-wide assays and indicates copy-number variations of chromosomal segments in a tumor genome. Specifically, chromosomal segments, deleted in a single or both copies of the diploid genomes of a group of cancer patients, point to location of tumor suppressor genes (TSGs) implicated in a cancer. We present a novel multipoint statistical method that is capable of estimating the location of TSGs by analyzing array-CGH data characterizing segmental deletions (hemi- or homozygous) in cancer genomes. Our approach is based on computing a multipoint score for intervals of consecutive probes that measures how likely it is for that genomic interval to be the TSG, sought after. A significance is given to the maximum scoring interval in a region using results from scan statistics. Based on these scores we can estimate the most likely left margin and right margin of the TSG interval. We assess the power of the method by examining how well it uncovers the true location of the TSG; we compute the Jaccard's measure of the overlap between the true location of the TSG and the estimated one. We applied our method to datasets simulated under different scenarios; each dataset contains 100 samples and the genomic region under investigation is of length 100 Mb with a probe every 5 kb; each dataset contains some samples that are sporadic and the rest are split between those with homozygous deletion and hemizygous deletion respectively of the TSG. In the table below we illustrate results on some of the important scenarios. For each scenario, we simulate 50 datasets and report the average overlap as given by the Jaccard's measure.

1-hit rate/sporadic rate	0%	50%
0%	0.72	0.48
50%	0.66	0.46

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Removing Ceiling Effect related Type II Errors in Two-locus Variance Component Linkage Analysis Identifies New QTLs for Serum Lipid Levels in Sib-pair Data. *M. Falchi[1,4], T. Andrew[1], H. Snieder[1,2], R. Swaminathan[3], G. Surdulescu[1], T.D. Spector[1].* 1) Twin Res & Gen Epidem Unit, St Thomas Hosp, London, United Kingdom; 2) Georgia Prevention Institute Department of Pediatrics, Medical College of Georgia, Augusta GA; 3) Dept of Chemical Pathology, Guys & St Thomas NHS Foundation Trust; 4) Medical Genetics Unit, Dept. Mother and Child, University of Modena and Reggio Emilia, Modena, Italy.

Given the underpowered sample sizes commonly used in human genetics linkage studies, minor quantitative trait loci (QTLs) effects often go undetected and those that are detected will be upwardly biased. We show that the discrepancy between the real biological and the estimated QTL effects are likely to often generate an unpredictable source of false-negative errors, reducing the power to detect multiple QTLs through linkage analysis. We apply a heterogeneity method to offset this problem in a two-locus setting, showing a greatly increased power to detect multiple QTLs both via simulations. The practical use of the new approach is illustrated by a genome-wide search (GWS) for serum lipid QTLs using a 10cM marker map for 961 female sib-pairs. Three genome-wide significant QTLs were identified for apolipoprotein A1 on chromosomes 8p21.1-q13.1 (LOD score 3.71), 9q21.32-33.1 (LOD score 3.28) and 10p15.1-p13 (LOD score 5.51), two for lipoprotein (a) on chromosomes 6q25.2-q27 (LOD score 10.18) and 21q21.1-q21.3 (LOD score 4.57) and two for triglycerides on chromosomes 4q28.3-32.1 (LOD score 3.71) and 5q23.1-q32 (LOD score 3.60). This approach has led to the identification of known and novel regions linked to serum lipid levels. The successful simultaneous QTL modeling method provides compelling evidence that the novel QTLs detected are likely to be reliable.

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The mannose binding lectin Arg52Cys gene coding variant uniquely predicts angiographic coronary artery disease. *M.J. Kolek[1], J.L. Anderson[1,2], J.F. Carlquist[1,2], B.D. Horne[1,3], T.L. Bair[1], J.L. Clarke[1], J.B. Muhlestein[1,2].* 1) Cardiovascular Department, LDS Hospital, Salt Lake City, UT; 2) Cardiology Division, University of Utah School of Medicine, Salt Lake City, UT; 3) Division of Genetic Epidemiology, University of Utah, Salt Lake City, UT.

The mannose binding lectin (MBL) gene encodes a soluble protein that binds potentially atherogenic *Chlamydia pneumoniae* and other bacteria, marking them for opsonization via complement. Several single nucleotide polymorphisms (SNPs) in the MBL gene are reportedly associated with lower plasma MBL levels and elevated coronary artery disease (CAD) risk. Patients entered into the Intermountain Heart Collaborative Study registry (1994-1998) who underwent coronary angiography were studied to assess the association of MBL SNPs with CAD prevalence (N=2,327). Plasma MBL was measured in a subset (n=302) and compared by genotype. Three coding (Arg52Cys, Gly54Asp, Gly57Glu), two promoter (G-550C and G-221C), and one 5' UTR SNPs (G+4A) were studied. Adjustments were made for CAD risk factors using logistic regression. Principal component analysis assessed the linkage disequilibrium (LD) between each SNP. Average age was 6412 years, 70% were male, and 77% had CAD. All 6 SNPs were associated with differences in plasma MBL (p0.03). The G-550C variant was in high LD with two other SNPs (G-221C and G+4A) that accounted for its variation, thus it was excluded from further analysis. G-221C and G+4A were not associated with CAD. Minor allele carriage in any one of the three exonic SNPs was confirmed to be associated with CAD (OR=1.32, p=0.020), as previously reported. However, only Arg52Cys was independently associated with CAD (adjusted OR=1.54, CI=1.07-2.21, p=0.019). Further, the dilution of risk that was found when all three exonic SNPs were jointly considered suggests that Arg52Cys may be the risk-related variant. Although all 6 SNPs were associated with plasma MBL, the Arg52Cys findings encourage exploration of the mechanisms underlying this SNPs effect on the MBL protein, including potential pro-atherogenic host response to infection.

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Subclinical Atherosclerotic Traits are Heritable: the Framingham Heart Study. *L.A. Cupples[1], C.Y. Liu[1,2], U. Hoffman[3], J.M. Massaro[1], S.J. Hwang[2], M.G. Larson[4], E. Manders[2], C.S. Fox[2], C.J. O'Donnell[2,3].* 1) Department of Biostatistics,Boston Univ Sch Public Health, Boston, MA; 2) National Heart, Lung and Blood Institutes Framingham Heart Study, Framingham, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Department of Mathematics and Statistics, Boston University, Boston, MA.

Coronary heart disease and other atherosclerotic cardiovascular diseases (CVD) comprise the leading causes of death for men and women in the U.S. Using noninvasive high resolution imaging modalities, subclinical atherosclerosis can be detected as quantitative levels of calcific deposits in major arterial beds such as the coronary arteries and aorta in large cohorts of men and women. Such traits have been reported to have genetic etiology. We studied coronary artery calcific deposits [CAC] and abdominal aortic calcification [AAC] measured between 2002 and 2005 by multidetector computed tomography in 2946 subjects from the second and third generations of 676 pedigrees of the Framingham Heart Study. The subjects ranged in age from 35 to 83 years (with the mean age at 51). We used variance component models in SOLAR to assess the heritability of the Agatston scores for both CAC and AAC. Each score was adjusted for gender, age, systolic blood pressure, smoking, total and HDL cholesterol, diabetes, body mass index, anti-hypertensive treatment, lipid treatment and prevalent CHD and CVD status using regression methods on traits derived from a logarithmic transformation after adding 1 to each value. The age-adjusted heritability of CAC is 304 percent and multivariable-adjusted heritability is 274 percent. For AAC, these estimates are 354 and 284 percent, respectively. Given these findings, genomewide linkage and association studies are warranted in order to identify important genomic regions in the pursuit of QTLs associated with the development of coronary heart disease.

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Phylogeny-Based Haplotype Association Analysis for Coronary Artery Disease and the Cholesteryl Ester Transfer Protein Gene. *B.D. Horne, J.F. Carlquist, J.L. Anderson, C.P. Mower, N.J. Camp.* Cardiology/Genetic Epidemiology, LDS Hospital, U Utah, SLC, UT.

Disease associations of candidate gene single nucleotide polymorphisms (SNPs) are notoriously difficult to replicate. Evaluation of haplotype groups based on phylogenetic SNP relationships is a novel approach that may increase study resolution. We evaluated the association to coronary artery disease (CAD) of haplotypes constructed from tagging SNPs (tSNPs) in non-recombinant regions of the cholesteryl ester transfer protein (CETP) gene. Non-diabetic, non-smoking patients (N=4811) with angiographically-significant CAD (70% stenosis) or normal coronaries (<10% stenosis) were genotyped for 11 tSNPs previously selected to represent 32 SNPs in the promoter, exons, and 3¢ UTR. Phylogenetic relationships were evaluated using PAUP and all CAD association analyses were multivariable-adjusted in logistic regression. Patient age averaged 6313 years and 63% were male. In the non-recombinant intron 8-3¢ UTR region, six terminal haplotype groups were found for 8 tSNPs, and association to CAD was found for haplotypes containing ATG (T+1086A, C+878T, G+2389A) with patients having 68%, 65%, and 60% CAD for carriage of 0, 1, and 2 haplotypes (for 2 vs 0 haplotypes: adjusted OR=0.58, 95% CI=0.34-0.99, p=0.046), for haplotypes containing AGGT (MspI, G+2389A, A373P, C+408T) with 67%, 67%, 63% CAD (2 vs 0: OR=0.76, CI=0.56-1.04, p=0.087), and for GGCT haplotypes in those 4 tSNPs with 67%, 70%, 70% CAD (1 or 2 vs 0: OR=1.34, CI=1.03-1.74, p=0.030). In the promoter-intron 8 region, 4 haplotype groups were found for 3 tSNPs (C-631A, Taq1B, and MspI), and for AAA haplotypes patients had 69%, 67%, 65% CAD (OR=0.78, CI=0.64-0.95, p=0.012). Evaluation of phylogeny-based haplotype groups in the CETP gene showed significant associations to CAD. For the ATG and AAA haplotype groups, the relative risks were similar to previous findings for T+1086A alone and for C-631A alone, respectively. However, for two other groups this phylogenetic grouping strategy extracted associations where single tSNPs did not. This suggests that phylogenic haplotype grouping may be used to guide and improve association analysis.

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Pleiotropic relationships between lipoproteins and two biomarkers of oxidative stress and inflammation in the San Antonio Family Heart Study. *D.A. Winnier[1], D.L. Rainwater[1], X-L. Wang[2], J.W. MacCluer[1], M.C. Mahaney[1].* 1) Southwest Foundation for Biomedical Research, San Antonio,TX; 2) Baylor College of Medicine, Houston, TX.

Recent research suggests that the association between variation in plasma lipoproteins and risk of cardiovascular disease is attributable in large part to the processes of oxidative stress and inflammation. We conducted statistical genetic analyses to characterize the relationships between circulating lipoproteins and two biomarkers of oxidative stress and inflammation: platelet activating factor acetylhydrolase(PAF-AH) and paraoxonase-1(PON1). From stored plasma samples for 1,406 San Antonio Family Heart Study participants, we measured PAF-AH (an LDL-associated phospholipase A_2 that degrades PAF); PON1 (an HDL-associated molecule that may inhibit LDL oxidation); and LDL, HDL, and their apolipoprotein (apo) components. We used a multivariate maximum-likelihood based, variance decomposition approach implemented in SOLAR to estimate the phenotypic, genetic, and environmental correlations between these traits. All traits had significant heritabilities (h^2= 0.23-0.77). As expected, each biomarker was more correlated to traits from its own lipoprotein moiety; thus PAF-AH exhibited significant correlations with LDL, its subfractions, and related apolipoproteins, the strongest of which was between PAF-AH and apoB ($_P$=0.53, $_G$=0.61, $_E$=0.47; P<0.02). We also observed significant correlations between PAF-AH and total LDL ($_P$=0.44, $_G$=0.43, $_E$=0.43). Between 20% and 36% of the additive genetic effects on PAF-AH and the LDL-related traits was due to pleiotropy. By contrast, PON1 exhibited weaker relationships with HDL and its related traits. We observed marginally significant correlations(P0.05) between PON1 and apoA-II ($_P$=0.28, $_G$=0.18, $_E$=0.47) and HDL-3 ($_P$=0.18, $_G$=0.17, $_E$=0.23),with shared genetic effects accounting for 18% or less of the genetic variance in these trait pairs. The actual genes responsible for these shared genetic effects on variation in lipoproteins and biomarkers of oxidative stress and inflammation-all recognized risk factors for CVD-may underlie variation in CVD risk as well.

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Stress-Induced Sodium Excretion, a New Heritable Intermediate Phenotype to Study the Genetics of Salt Sensitive Hypertension? *D. Ge, H. Zhu, Y. Dong, X. Wang, G.A. Harshfield, F.A. Treiber, H. Snieder.* Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, Bldg HS-1640, Augusta GA 30912, USA.

We have recently proposed that impaired stress-induced pressure natriuresis, measured by urinary sodium excretion ($U_{Na}V$), is an indication of salt sensitivity. However, it is unknown whether and to what extent genetic factors contribute to stress-induced $U_{Na}V$. To assess the heritability of stress-induced $U_{Na}V$, we studied 199 African- (AAs) and 245 European-American (EAs) twin pairs from the South-Eastern US, including monozygotic and dizygotic of same- as well as opposite-sex (mean age: 17.6 3.3; range: 11.9-30.0). We used a combination of an overnight and a pre-stress $U_{Na}V$ as the baseline $U_{Na}V$, while the stress $U_{Na}V$ was measured after a protocol that included an echocardiographic examination and exposure to three stress tasks: a social stressor interview, a virtual reality car driving test and a competitive video game. All univariate and multivariate genetic model fitting was performed using Mx software. No significant sex effects on genetic and environmental contributions to $U_{Na}V$ were detected. The best fitting univariate model showed that the heritabilities [h^2 (95% confidence interval)] for stress $U_{Na}V$ were 0.41 (0.23-0.56) in EAs and 0.53 (0.35-0.66) in AAs, but the difference between the two ethnic groups was not statistically significant. A multivariate model was then used to estimate the genetic and environmental influences shared with baseline $U_{Na}V$, showing only 23% (14-32%) and 13% (6-29%) of the total variance could be attributed to shared factors in EAs and AAs, respectively. The specific heritabilities for stress-induced $U_{Na}V$ were 0.30 (0.09-0.47) in EAs and 0.53 (0.33-0.67) in AAs after the adjustment for the shared influences with baseline $U_{Na}V$. In summary, this study shows stress-induced $U_{Na}V$ is a heritable phenotype that can be used to study the genetic etiology of salt sensitivity in relation to hypertension in both AA and EA populations.

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Seeing the Forest for the Trees in Genome-wide Association Studies. *J. Witte, E. Jorgenson.* Epidemiology & Biostatistics, Univ California,San Francisco, San Francisco, CA.

Advances in genotyping and greater knowledge about human genetic variation have led to an escalating interest in genome-wide association studies. Undertaking these studies requires large SNP sets with comprehensive coverage of either the entire genome or at least all known genes (i.e., positional cloning or candidate gene based sets). We first consider the rationale for developing such SNP sets, and the resulting implications for genome-wide association studies. We then compare the sample sizes needed forand relative cost ofpositional cloning versus candidate gene studies. The positional cloning approach requires substantially more SNPs, slightly larger sample sizes, and thus is considerably more expensive to perform than the candidate gene approach. However, the candidate gene approach may miss a limited number of causal variants outside of genes, specifically variants in *cis*-regulatory regions which may be located far away from coding regions. The expected ratio of causal variants in genes versus outside of genes helps distinguish which approach is most appropriate.

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A realistic power estimation for genome-wide association tests using the Phase I HapMap data set. *K. Nakazaki[1], Y. Nannya[1], M. Kurokawa[1], S. Chiba[1, 2], S. Ogawa[1, 3, 4].* 1) Departments of Hematology/Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Cell Therapy/Transplantation Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Regeneration Medicine for Hematopoiesis, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 4) Japan science and technology agency, Basic research programs,'@CREST, Saitama, Japan.

With recent advances in high-throughput SNP typing technologies, genome-wide association studies involving a large number of SNP markers have become a more feasible approach to identify causative genes for common diseases of complex genetic traits. It is of particular importance to evaluate the expected genetic power before conducting such studies requiring costly large-scale genotyping. However, it is still unclear how much power could be obtained with a particular set of SNP markers used. While several estimations have been made for power of association studies, they do not take into account the strength of association of the target SNP with SNPs included in a given marker set. It is of particular importance to evaluate the expected genetic power before conducting such studies requiring costly large-scale genotyping. The recent publication of the phase I data from the international HapMap project, allows us to more accurately calculate parameters of linkage disequilibrium between a given marker within the subset of these HapMap SNPs and a randomly selected candidate for the disease SNP, thus enabling us to better estimate the probability of finding possible association for a particular marker set. Here we applied this method of power calculation to the Affymetrix 100K mapping set and provide a gross estimation of the power that could be obtained when the 100K set is used for association analysis. We also show the effects of SNP marker selection on the power of association tests. Our method, which is implemented in the PowerSNP program, will provide a useful tool for evaluating the performance of a given subset of the HapMap SNPs and also for constructing an optimized marker set for genome-wide association studies.

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Simulation study of empirical NULL distribution of LODPAL's LOD scores. *N. Mukhopadhyay[1], B.H. Reck[1], D.E. Weeks[1,2].* 1) Dept Human Genetics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA; 2) Dept of Biostatisics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA.

Several studies have noted that the logistic regression model used by LODPAL produced LOD scores with higher false-positive rates than expected. However, no methodical study has been made of this inflation. Covariate-based methods are becoming increasingly popular in mapping complex diseases, hence, such a study is of great interest.Our simulation study is designed to systematically characterize this inflation.Here, we examine the empirical null distributions of LODPALâs LOD-scores by vary ing the following study parameters: (a) number of affected sibling-pairs in a pedigree, (b) marker densities, (c) number of covariates, and (d) degree of inter-sibling as well as trait-covariate correlation. Our aim is to study the effects of varying these parameters on (i) thresholds for suggestive and significant linkage, and (ii) tails of empirical null distributions as sample size increases.Phenotypes for sibships of size 5 were created as follows. A binary trait and 4 covariates were created for each sibling. The binary trait was simulated using a continuous underlying liability phenotype from a multivariate normal distribution, and taking the upper 75th percentile to be affected. Then we applied an ascertainment step, selecting samples of 50, 100, 150, and 200 sibships with 2,3,4 and 5 affected siblings respectively. Similarly, each of the 4 covariates for the 5 siblings were drawn from a multivariate normal distribution.There is no correlation among the covariates. We used a low value (0.2) and a high value (0.7) for inter-sibling and trait-covariate correlations. We studied all four combinations of these correlation values. Genotypic data consists of 11 markers with realistic frequencies, and with map spacings of approximately 1, 5 and 10 cM. Genotype data were simulated under the assumption of no linkage to either the trait or any of the covariates. 10,000 replicates of genotype data were simulated for each sibship-types(2,3,4,5 affecteds), map spacing (1,5,10 cM), and sample size (50,100,150, 200). LODPAL was run on each simulated set with 0,1,2,3,and 4 covariates.

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Breaking very large complex pedigrees and the impact on genetic analysis. *D. Brocklebank, J. Gayan, L. Cardon.*
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Linkage based analyses employing large extended pedigrees represent an invaluable tool for localizing and identifying human quantatitive trait loci. However, even with the substantial advances in statistical modelling, incorporated in packages such as Simwalk and LOKI, pedigrees of large size and high levels of complexity are often beyond current constraints. This generally requires breaking such pedigrees into smaller discrete subpedigrees and losing potentially valuable information. We have developed novel methods to efficiently encode and process arbitrarily large and complex pedigree structures employing concepts from graph theory. Algorithms are implemented for pedigree management, pruning, clustering subpedigrees, quantifying pedigree relationships and performing variance components analysis. With the aim of maximising extraction of pedigree information for analysis, we present systematic pedigree partitioning techniques to identify subpedigrees of computationally manageable sizes. Different and complimentary techniques are suggested for pedigrees of varying sizes, including a method based on kinship information for very large structures, and methods based on sampling connecting individuals for removal. We have developed automated tools to identify the approach optimal for a given pedigree structure. We apply methods to real extended pedigrees, including the Tristan de Cunha pedigree, a highly inbred connected pedigree comprising 363 individuals and containing more than 100 loops. Through simulation of phenotypic and genotypic data to reflect the true data patterns, we investigate the impact of varying degrees of pedigree breakage on the accuracy of Identity By Descent (IBD) computations, and the effect of resulting bias in IBD estimates on the power to detect linkage under various genetic models. IBD estimates are obtained using both Markov Chain Monte Carlo (MCMC) methods and deterministic methods where computationally feasible. For larger sub pedigrees, the MCMC estimates are compared with true IBDs, and the effect of incorporating increasingly distant ancestral information on the accuracy and convergence of MCMC estimates is explored.

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Computing power in genetic case-control association studies in the presence of phenotype and genotype misclassification errors. *F. Ji[1], Y. Yang[2], C. Haynes[1], D. Gordon[1].* 1) Laboratory of Statistical Genetics, Rockefeller University, New York, NY; 2) University of Science and Technology of China, Hefei, China.

It is well established that phenotype and genotype misclassification errors reduce the power to detect genetic association. Resampling a subset of phenotype and/or genotype data (i.e., double-sampling) with a gold standard measurement is one method to address this issue. We derive the non-centrality parameter (NCP) for the recently published Likelihood Ratio Test Allowing for Error (LRTae) in the presence of random phenotype and genotype errors. With the NCP, power and sample size can be analytically determined at any significance level. We verify analytic power with simulations using a 2^k factorial design given high and low settings of: case and control genotype frequencies, phenotype and genotype misclassification probabilities, total sample size, ratio of cases to controls, and proportions of phenotype and/or genotype double-samples, at significance level settings of 0.05, 0.01, 0.001, and 0.0001. For 0.05 and 0.01 significance levels, the absolute difference in power between computer simulation and theoretically determined results is at most 0.016. For the 0.001 and 0.0001 significance levels, 2% (respectively, 5%) of the settings have a difference larger than 0.02. ANOVA analysis indicates that differences are mainly affected (in order of F-statistic) by the minor allele frequency, ratio of cases to controls, and total sample size. Our method enables researchers to perform power and sample size calculations in the presence of phenotype and genotype errors and also to perform cost/benefits analysis using different experiment designs (e.g., larger sample sizes with no double-samples vs. smaller sample sizes with some double-samples) without the need for time-consuming computer simulation.

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Mutual Information Relevance Networks for Modeling Gene-Gene. *L. Luo[1], Y. Wang[1], L. Jin[1,2], M. Xiong[1,3].* 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Center for Genome Information, University of Cincinnati, Cincinnati, OH, USA; 3) Human Genetics Center, University of Texas - Houston Health Science Center, Houston, TX, USA.

Shannon entropy, originally defined in information theory (Shannon 1948), is used to measure the uncertainty removed or the information gained by performing an experiment and to measures the uncertainty of random variables or the degree of nonstructure of a system. When it is applied to characterize DNA variation, entropy measures genetic diversity and extracts the maximal amount of information on a set of SNP markers. The entropy can quantify the level of the linkage disequilibrium (LD) between two marker loci. It is known that gene-gene interaction will create LD between two interacted genes. Intuitively, we can test interaction between two genes by comparing the difference in the levels of the LD between two genes in the cases and controls. In this report, we propose to use entropy and mutual information to model interactions between genes. A higher mutual information between two genes indicates that one gene is more likely to be non-randomly associated with another gene. By comparing mutual information for a pair of genes in cases and controls we can measure the strength of interaction between genes. Therefore, by calculating pair-wise mutual information, we can link interacted genes and remove uninteracted genes to generate mutual information relevance networks, which can be used to model genetic interaction networks.

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Estimate Genotyping Error from Multi-locus Genotype Data of Unrelated Individuals. *G. Zhang[1], R. Chakraborty[1], L. Jin[1,2].* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Institute of Genetics, Fudan University, Shanghai, China.

Haplotype analyses and linkage disequilibrium (LD) mapping of complex diseases depend crucially on the accuracy of genotype data. Even moderate genotyping error rates can have serious affects on haplotype or LD measure inferences. Unlike pedigree data, in which genotyping error can be detected through Mendelian incompatibilities or close double recombinants, in unrelated individuals, genotyping error of single marker can only be partially inferred from deviation of Hardy-Weinberg equilibrium. In this presentation, we describe an expectation-maximization (EM) algorithm to detect genotyping errors by jointly analyzing genotype data of multiple markers in close linkage disequilibrium. Specifically, the algorithm estimates the haplotype frequencies conditional on multi-locus genotype data and an assumed (priori) model of genotyping error probabilities, and calculates the posterior genotyping error probabilities based on the observed genotype data and the estimated haplotype frequencies. Through simulation study, we examine the accuracies of our proposed method on genotyping error detection and haplotype frequency inference, and explore the effects of marker density, genotyping error rate and sample size on this method. The results show that our method performs well in estimating genotyping errors and haplotype frequencies, especially for multiple markers in high LD.

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Power to detect imprinting using linkage data: Parent-of-origin penetrance vs. independent male-female recombination fractions. *D.A. Greenberg[1], C. Monti[1,2], B. Feenstra[1,3].* 1) Div Stat Genet, Dept Biostat, Columbia Univ, NY, NY; 2) Dept. Applied Health Sci, Pavia Univ, Italy; 3) Royal Vet & Agricult Univ, Dept Natural Sci, Denmark.

There are an increasing number of genes known whose expression depends on the sex of the parent contributing the gene, i.e., imprinting. For brain-related traits, this may be particularly important; in mice, there is region-specific imprinting of some genes. We tested two methods for detecting imprinting: A) lod score maximization w.r.t. parent-of-origin-based penetrances; and B) lod score maximization w.r.t independent male-female recombination fractions.

We simulated family data with imprinting, which we defined as existing when the penetrance in offspring depended on the parent of origin of the allele. We maximized the two-point lod score: a) over parent-of-origin penetrances (PP) and b) over combinations male-female recombination fraction (RF). In both cases, we compared the max score assuming equal parameter values from mothers and fathers to the global max over all combinations. Only data sets in which evidence for linkage was significant were used to calculate power to detect imprinting. For both approaches, we calculated the chi-squared value based on lod score differences to determine the significance for imprinting. We calculated the power over a range of data set sizes. We also tested the confounding effects of 1) reduced penetrance, 2) heterogeneity, 3) differential male-female penetrance, and 4) biased ascertainment.

If neither heterogeneity nor reduced penetrance is present, the PP method has more power to detect imprinting than the RF. Imprinting detection power actually increases for the PP method if reduced penetrance exists, given linkage is detectable. However, the power to detect imprinting increased for the RF method and decreased for the PP method when heterogeneity existed. If one sex had higher penetrance than the other, the PP method could falsely indicate imprinting, while the RF method was not fooled. Neither method gave biased results because of parent-specific ascertainment bias.

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An S-Plus/R library for Quantitative Trait Linkage Analysis. *M. de Andrade[1], E. Lunde[1], E.J. Atkinson[1], J. Chen[2], C.I. Amos[2].* 1) Div Biostatistics, Mayo Clinic, Rochester, MN; 2) Dep Epidemiology, UT M.D. Anderson Cancer Center, Houston, TX.

We have implemented the Multic program from the ACT software in an S-Plus/R library called multic. Multic estimates variance components for quantitative linkage analysis using maximum likelihood method (scoring algorithm) for multivariate traits and longitudinal data. Additionally, we have created tools to import SIMWALK2 and Solar IBD data to be used by multic. The S-Plus/R library provides tools to easily run the main analysis, summarize the results for polygenic and major gene models, and plot the overall results. Diagnostic tools are also provided to detect influential families or individuals. The integration of multic in S-Plus and R provides the users with all the niceties already available in these software packages such as the usage of modeling language to create transformations and interactions, the ability to use results from other models (like factor analysis) as potential traits or covariates, and a suite of sophisticated graphics tools. We will present examples of the proposed features and will include runtime comparisons of SOLAR with Multic in its former format and with Multic embedded in S-Plus/R. The software will be publicly available by the time of the meeting, with user-friendly documentation.

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SimPed: A Simulation Program to Generate Haplotype and Genotype Data for Pedigree Structures. *S.M. Leal[1], K. Yan[1], B. Müller-Myhsok[2].* 1) Department of Molecular and Human Genetic, Baylor College of Medicine, Houston Texas, USA; 2) Computational Genetics Group, Max-Planck-Institute of Psychiatry, Munich, Germany.

With the widespread availability of SNP genotype data, there is greater interest in analyzing pedigree haplotype data. For microsatellite markers due to their physical distance, intermarker linkage disequilibrium is usually low; however, for dense maps of SNP markers, there can be strong linkage disequilibrium between marker loci. Linkage analysis (parametric and nonparametric) and family-based association studies are currently being carried out using dense maps of SNP marker loci. Given the widespread use of dense maps of marker loci, it is important to be able to generate a large number of marker loci in linkage disequilibrium or a mixture of markers with some loci in linkage disequilibrium and others in linkage equilibrium for pedigree data. In order simulate this type of data the SimPed program which is written in C was developed. It can quickly generate haplotype and/or genotype data for pedigrees of virtually any size and complexity. Marker data either in linkage disequilibrium or equilibrium can be generated for > 10,000 diallelic or multiallelic marker loci. Haplotypes and/or genotypes are generated for pedigree structures using specified genetic map distances and haplotype and/or allele frequencies. SimPed quickly generates data, for example, genotypes for 50 marker loci defining a total of 20 haplotypes were generated for a three-generational pedigree with 16 family members. For this study, 1,000 replicates were generated in 0.73 seconds on a computer with a Xeon 3.0 GHz processor and 4 GB of RAM running under Red Hat (v9.0) Linux operating system. Additionally, for the same pedigree structure the genotypes for 50 SNP marker loci were generated for 1,000 replicates in 0.75 seconds. The data which SimPed generates is useful for a variety of analysis purposes, including evaluating methods that estimate haplotype frequencies for pedigree data, evaluating type I error and estimating empirical p-values for linkage and family-based association studies.

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Simulation and analysis of complex multifactorial diseases. *M.A. Schmidt, E.R. Hauser, E.R. Martin, S. Schmidt.*
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Identifying genes contributing to complex multifactor diseases of genetic and environmental origin is one of the biggest challenges in genetics. Statistical software is available to address some of these complexities, however simulation studies of new software often focus on only a few selected data generation mechanisms. Thus it is important to give investigators the tools to test new software under a variety of conditions. We have developed a general simulation package called SIMLA, which can simulate a great variety of complex multifactorial disease models and provides many input file formats for commonly used analysis programs. SIMLA can simulate up to two disease loci on either X-linked or autosomal chromosomes, distinct relative risk parameters for males and females, up to two environmental covariates, each of which may be correlated within families, and linkage disequilibrium of marker and disease loci. Various ascertainment criteria for the data set can be specified. Genes and environmental covariates may interact in a number of ways, as specified by highly flexible penetrance functions. SIMLA provides the user with a number of summary statistics for the simulated data, such as observed IBD-sharing, allele and environmental exposure frequencies in selected individuals, sibling recurrence risk and penetrance values for all exposure combinations. With these statistics, the user may evaluate different data generation mechanisms with standard measures of genetic effect sizes. The number of markers, print options, disease prevalence and ascertainment criteria impact the performance of SIMLA, but it can typically generate more than 100 pedigrees per second with a 100-marker map on common hardware. This immediate feedback about the impact of various factors on the generated data sets, prior to analyzing them with any particular statistical-genetic software, makes SIMLA particularly useful. Several plausible disease models and associated analysis methods have been introduced in the literature (Marchini et al. 2005; Ritchie et al. 2001). We will refer to these models and illustrate how to implement them using the SIMLA software.

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Independent population based studies support the role of Upstream Stimulatory Factor 1 (*USF1*) -gene variants in coronary heart disease. *M. Perola[1], K. Komulainen[1], K. Ahonen[1], M. Alanne[1], J. Saarela[1], S. Kulathinal[2], K. Kuulasmaa[2], K. Silander[1], V. Salomaa[2], L. Peltonen[1].* 1) Dept. of Molecular Medicine, KTL, Biomedicum, Helsinki, Finland; 2) Dept. of Epidemiology and Health Promotion, KTL, Mannerheimintie 166, 00300, Helsinki, Finland.

The *USF1* gene, encoding a transcription factor regulating several genes of lipid metabolism, has been associated with familial combined hyperlipidemia (FCHL), and various other trait components predisposing to coronary heart disease (CHD). We studied here the allelic variants of the *USF1* gene in two independent population-based cohorts, FINRISK 92 of 5999 Finns and FINRISK 97 of 8141 Finns, to assess the significance of the gene as a risk factor for CHD at the population level. Five haplotype-tagging SNPs across the 5.7 kb *USF1* gene were genotyped in a case cohort setting for a total of 2222 individuals including 843 CHD cases. Importantly, the gene was found to be associated with the risk of CHD in both cohorts.

We initially explored for association between the *USF1* gene and the prospective risk of CHD in FINRISK 92 cohort with a follow-up time of 10 years using the Cox proportional hazards analysis. We established an association between the risk of CHD in females and *USF1* risk allele defined by five SNPs (HR 5.8, 95% Cl 1.7-19.3, P=0.005). We also identified a putative protective role of another five SNP allelic haplotype (HR 0.13, 95% Cl 0.03-0.50, P=0.003). Comparable evidence of the effect of the *USF1* gene was obtained from the analysis of FINRISK 97 females (HR 4.3, 95% Cl 1.2-15.6, P=0.024 and HR 0.47, 95% Cl 0.16-1.45, P=0.19 for a risk and protective allele, respectively). Combined analysis of the two cohorts further supported the findings (HR 3.7, 95% Cl 1.7-8.0, P=0.001 and HR 0.34, 95% Cl 0.16-0.72, P=0.005 for the risk and protective allele, respectively). These results underline the importance of the *USF1* gene variants as a CHD risk factor at the population level. The critical function of *USF1* in the lipid metabolism well agrees with its important role in the pathogenesis of CHD.

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Genetic variations in thrombosis cascade predispose to cardiovascular events in a prospective follow-up study. *K. Ahonen[1], K. Komulainen[1], M. Alanne[1], K. Silander[1], K. Kulathinal[2], V. Salomaa[2], L. Peltonen[1,3], M. Perola[1,3].* 1) Dept. of Molecular Medicine, KTL, Haartmaninkatu 8, Helsinki, Finland; 2) Dept. of Epidemiology and Health Promotion, KTL, Mannerheimintie 166, Helsinki, Finland; 3) 3Dept. of Medical Genetics, University of Helsinki, Haartmaninkatu 8, Helsinki, Finland.

Thrombomodulin (TM), intercellular adhesion molecule -1 (ICAM1), protein C (PROC) and factor V (F5) are critical elements of a natural clotting cascade, where TM activates PROC, which leads to inactivation of F5. Previous studies of single polymorphisms of these four genes have suggested association to cardiovascular diseases (CVD). To address the details of genetic variation of this physiological cascade and its role in pathogenesis of CVD, we analyzed altogether 23 single nucleotide polymorphisms (SNPs) of these four genes in a prospective follow-up study. FINRISK92 is a stratified random sample of men and women aged 25-64 years examined in 1992. The follow-up time for CVD events was ten years (1992-2001). From the total sample of 5,999 individuals, 999 were chosen for genotyping using case-cohort study design. 222 persons had a history of cardiovascular events at the baseline. During the follow-up, 195 incident coronary events (CHD) and 68 ischemic stroke events (CI) were observed. The SNPs were selected to monitor the allelic diversity by capturing the major haplotypes. All the SNPs were tested in Cox proportional hazards model using CHD, CI and all CVD events as endpoints. Of the five PROC SNPs analyzed, two showed association to CI in the whole genotypic sample (HR 3.43, CI 95% 1.43-8.21, p=0.0057 and HR 3.18, CI 95% 1.31-7.64, p=0.011). For all CVD events, an association was observed with one SNP of PROC in men (HR 2.25, CI 95% 1.15-5.50, p=0.020) and one ICAM1 SNP in women (HR3.98, 95% CI 1.19-13.36, p=0.025). Several F5 SNPs showed association to CHD in females, whereas TM SNPs showed no association. Our results imply that genetic variation in this cascade contribute to the risk of cardiovascular events. Future analyses focus to define the epistatic character of the genes.

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The Burden of genetic disease in a hospitalized pediatric population in Colombia. *I. Zarante, P. Paez, N. Garcia, F. Suarez.* Inst de Genetics Humana, Pontificia Univ Javeriana, Bogota, Colombia.

The important role of genetics in pediatric illness has been increasingly recognized. There is not information about the genetic burden in disease affecting children in developing countries like Colombia. The purpose of this research is to establish the frequencies of diseases with clearly established genetic etiology and those with predisposition or susceptibility in a hospitalized pediatric population at a Universitary Hospital in 2003. The diagnosis database of all hospitalized children during 2003 from one to eighteen years old from the Hospital Statistic Department was recorded. With the objective of determine the genetic basis of the diseases affecting children, the diagnosis were assigned to the following categories: Monogenic disease with clearly monogenic inheritance (I), chromosomal diseases (II), complex disease conditions including multifactorial diseases and malformations (III), unknown etiology disease but with probably genetic basis (IV), diseases without genetic cause including infections and trauma (V), unclassified diagnosis (VI). Delivery, pregnancy, related pregnancy disease and related neonatal diagnosis were excluded. A total of 3901 records were analyzed. 1796 were excluded. A diagnosis category assignment was reached in a total of 2105 records. Sex ratio male: female were 1,5:1. The age range was 1 to 18 years with an average of 6.7 (5,3). The major percentage of diagnosis were in category III (27%) and V (13,2 %) which are similar percentages reported in other similar works performed in developed countries. Other major causes of hospitalization were category IV (13.1%) which included diseases like leukemia, malignat long bone tumors, depressive episodes and epilepsy. The commonest diagnoses were respiratory tract infections in category V (55.3%). The commonest malformation were congenital heart defects. This work indicates that 30% of the disease in this hospitalized pediatric population has a strong genetic etiology similar to the reported in developed countries, Colombia has achieved a significant epidemiological transition but still a lower resource country that need to develop a complete medical genetic service.

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Identification of Genetic Interaction Networks. *Y. Wang[1], J. Zhao[2], X. Zhou[3], W. Wang[4], J. Li[1], M. Xiong[2].* 1) Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas Health Scinece Center at Houston; 3) Medical School, University of Texas Health Science Center at Houston; 4) Chinese National Human Genome Center at Shanghai.

Current research demonstrates that only a small proportion of the disease risk is due to the influences of variations in a single gene with large genetic effects. In the real world, complex diseases develop as a consequence of interactions between multiple DNA variants and exposures to environmental agents varying over time and space, which are organized into networks. Actions of genes through networks are a consequence of complex molecular interactions occurring during biological processes such as metabolism, transcription, signal transduction and translations. The phenotypic variations depend on the genetic interaction networks. Dissecting genetic architecture of complex diseases requires uncovering hierarchically organized gene interaction networks. Unfortunately, the statistical methods for reconstruction of genetic interaction networks have not been well developed. Therefore, developing statistical methods for studies of genetic interaction networks is urgently needed. In this report, we present a general statistical framework for identifying genetic interaction networks and computational algorithms for discovering structure of genetic interaction networks. The identified genetic interaction networks will be compared with regulatory networks, protein-protein interaction networks and metabolic networks. . The proposed statistical methods and computational algorithms will be applied to atherosclerosis disease study.

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Differential Prediction of Coronary Artery Disease among Diabetics by CETP Tagging SNPs. *C.P. Mower[1], N.J. Camp[2], B.D. Horne[1,2], J.L. Anderson[1,2], J.L. Clarke[1], M.J. Kolek[1], J.T. McKinney[3], J.F. Carlquist[1,2].* 1) CV Research, LDS Hospital, SLC, UT; 2) Genetic Epidemiology and Cardiology Divisions, U of U, SLC, UT; 3) Idaho Technology, SLC, UT.

The cholesteryl ester transfer protein gene (CETP) regulates plasma lipid fractions and HDL cholesterol, but its effect on coronary artery disease (CAD) in the presence of diabetes mellitus (DM) may differ. The use of tagging single nucleotide polymorphisms (tSNPs) allows for better replications of SNP analyses. We evaluated the association between CETP tSNPs (C-631A, Taq1B, MspI, F287F, T+1086A, C+878T, G+2389A, A373P, C+408T, NlaIII, A+1825C) and CAD in the context of DM. DM (n=1273) and non-DM patients (n=4812) undergoing coronary angiography and free of any smoking history were included. Significant CAD (70% stenosis) was compared to non-CAD (10% stenosis) for the 11 tSNPs previously chosen for CETP. Genotyping was performed by quantitative PCR with probe hybridization. Logistic regression adjusted for traditional risk factors. CAD was present among 85% of DM and 66% of non-DM. DM patients averaged 6412 years of age and 40% were female, and only G+2389A (intron 10) had $p<0.25$ for association to CAD (86%, 85%, and 79% CAD for GG, GA, and AA). With full adjustment, AA vs. GG had OR=0.59 (95% CI: 0.36,0.97, p=0.036). To confirm this finding, a quasi-validation analysis predicted the presence of DM among CAD patients (age: 6612 years, female: 28%) and separately among those with no CAD (age: 5614 years, female: 45%). DM was less prevalent for the +2389 AA genotype among CAD patients (DM: 27%, 24%, 23% for GG, GA, AA), but was more prevalent for non-CAD patients (10%, 11%, 14% for GG, GA, AA), validating the CAD prediction results among DM (AA vs. GG: p-interaction with CAD= 0.022). CETP tSNP G+2389A (tagging the intron 8-exon 16 region) predicts CAD in DM, although it was found previously to not predict CAD among non-DM. This was validated, predicting differences in DM when stratified by CAD status. This study suggests that genetic risk for CAD among DM may involve similar pathways as in non-DM, but that the specific mechanisms may differ, and this hypothesis requires further testing.

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Extended Family Investigation of Nephropathy and Diabetes (E-FIND): Design and conduct of genetic studies. *N. Arar, R. Duggirala, H. Abboud.* Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX.

The Family Investigation of Nephropathy and Diabetes (FIND) study is designed to localize susceptibility genes for diabetic nephropathy (DN) by performing full genome scan in DN probands, their diabetic siblings with and without DN. As one participating center in FIND we recruited 300 DN probands and 900 siblings. The Extended Family Investigation of Nephropathy and Diabetes (E-FIND) is a subproject of the FIND. The goal of the E-FIND is to establish a pedigree-based genetic study by enrolling 50 multiplex Mexican American families of FIND probands 1st, 2nd, and 3rd degree relatives in order to assess susceptibility genes for DN using a variance components linkage approach. Another goal is to identify strategies to improve informed and voluntary participation in genetic family studies (GFS) among minority populations. Method: Structured interviews with enrolled subjects were conducted to (1)gather information about the family history of diabetes and DN, and (2)collect clinical data related to diabetes and DN using the FIND medical questionnaires. A separate set of questions assessed subjects informed and voluntary participation in GFS using the Contextual Assessment Questionnaire developed and validated in our center. Urine and blood samples were collected from all enrolled subjects for phenotype assessment regarding diabetes and DN. Additionally, buffy coat will be isolated from all blood samples for future DNA analysis. Results: We contacted 137 individuals representing 21 FIND families. Of those, 114 subjects were willing to participate in the E-FIND study, while 23 refused. Enrollment rate of 83.2% is appropriate to build pedigree-based FIND families and achieve the required sample size to perform linkage analysis. Based on our experience in the FIND and E-FIND studies, we will discuss the: (1) rationale for complex pedigree-based design as compared to sib pair linkage approach to search for DN genes, (2) methods for screening, contacting and enrolling subjects in GFS, (3) ethical issues associated with subjects participation in GFS and effective strategies to address them. Support/NIH/NIDDK; George M. O' Brien Kidney Research Center.

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Comparison of Methods for Stratified Analysis of Association Studies in Fine-Mapping Type 2 Diabetes in Multiple Populations for Chromosome 1q. *R. Hanson[1], E. Zeggini[2], W. Rayner[2], B. Mitchell[3], J. O'Connell[3], A. Shuldiner[3], S. Elbein[4], M.C.Y. Ng[5], K. Xiang[6], P. Froguel[7], P. Deloukas[8], M. McCarthy[2], W. Knowler[1], International Type 2 Diabetes 1q Consortium.* 1) NIDDK, Phoenix, AZ; 2) Oxford University, Oxford, UK; 3) University of Maryland, Baltimore, MD; 4) University of Arkansas, Little Rock, AR; 5) Chinese University of Hong Kong, Hong Kong, China; 6) Shanghai Diabetes Inst, Shangai, China; 7) CNRS UMR 8090, Lille, France; 8) Wellcome Trust Sanger Inst, Hinxton, UK.

Methods for pooling results for genetic association studies include the Mantel method, which estimates a common effect across populations, and Fishers method, which does not. We compared these methods in a large fine-mapping study of type 2 diabetes in a 13.5 Mb region on chromosome 1q. Data include 2331 SNPs from a total of 1617 diabetic cases and 1640 nondiabetic controls from 7 different populations. The Mantel extension test (^2_M) was applied to estimate the common effect of genotype on diabetes across all populations, under an additive model, and to assess its statistical significance. P-values from the test for trend in each population were also combined using Fishers method and the 1 df equivalent ^{2} for the resultant p-value was taken (^2_F). For each SNP, a permutation procedure was used to assess whether the difference (^2_M-^2_F) departed from that expected under no association. The rank correlation between p-values for the two methods was 0.37. 42 SNPS from 8 regional clusters were associated with diabetes at $p<0.001$ by at least 1 method. For 36 of these, ^2_M gave stronger evidence for association than did ^2_F ($p<0.0001$ by sign test). However, in 1 region, where different alleles were associated in different populations, ^2_F gave much stronger evidence and ^2_M-^2_F was lower than expected by chance ($p=0.0004$). The Mantel and Fisher methods, although correlated, provide complementary information. The Mantel method gives stronger results in most significant SNPs, suggesting effects are homogeneous across populations. Regions where Fishers method gives stronger results may reflect allelic heterogeneity or different linkage disequilibrium across populations.

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Variants in the HNF4A gene associated with diabetes and related traits in Hispanic Americans: The IRAS Family Study. *T.E. Fingerlin[1], C.D. Langefeld[2], A.B. Lehtinen[2], S.S. Rich[2], R.N. Bergman[3], S.M. Haffner[4], M. Bryer-Ash[5], D.W. Bowden[2], J.M. Norris[1].* 1) University of Colorado at Denver and Health Sciences Center; 2) Wake Forest University School of Medicine; 3) University of Southern California; 4) University of Texas Health Sciences Center; 5) University of California Los Angeles.

Variation in the HNF4A gene has recently been associated with type 2 diabetes (T2D) in Finnish, Ashkenazi Jewish and several other populations. We typed 23 SNPs spanning the HNF4A gene in 842 Hispanic American (HA) individuals in 45 families and 310 African American (AA) individuals in 21 families in the IRAS Family Study (IRASFS). We tested for association between these SNPs and T2D using the pedigree disequilibrium test. We identified several SNPs in and near the P2 promoter significantly associated with T2D in the HA sample (rs4810424 [p=.007], rs1884613 [.009], rs1884614 [.008], rs2144908 [.008], rs6073418 [.03]). We did not detect association with any SNPs in the AA sample; SNPs rs1884614, rs2144908 and rs6073418 had p=.09 for the putative at-risk alleles, suggesting that power in the smaller AA sample may be an issue. Minor allele frequencies for SNPs rs1884614 and rs2144908 were more common in our HA pedigrees (.47 and .46) and less common in our AA pedigrees (.12 and .12) compared to published Caucasian (C) samples (.17-.20). Thus, haplotypes with the at-risk allele at each of the SNPs are more (less) common in our HA (AA) sample than in published C samples. We also tested for association between these markers and glucose homeostasis measures from an FSIGT. Using a GEE1 approach, we found modest association between P2 promoter SNPs and SI in AA (p=.02-.03), but no evidence for association with acute insulin response or disposition index. In summary, we observed associations between markers in the P2 promoter region of HNF4A and T2D in an HA sample. Preliminary results suggest that these SNPs may contribute to variation in insulin resistance, but not disruption of insulin secretion. We are currently testing these results in a second set of independent families from the IRASFS in an attempt to corroborate these findings.

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Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1727 healthy Caucasians. *I.M. Heid[1], S.A. Wagner[1,2], H. Gohlke[1], B. Iglseder[2], J. Mueller[3], P. Cip[2], G. Ladurner[2], R. Reiter[2], A. Stadlmayr[2], T. Illig[1], F. Kronenberg[4], B. Paulweber[2].* 1) GSF-National Research Center, Neuherberg, Germany; 2) Paracelsus Private Medical University Salzburg, Austria; 3) Technical University, Munich, Germany; 4) Innsbruck Medical University, Innsbruck, Austria.

Reports on variants of the adiponectin (APM1) gene showed numerous, but inconsistent associations with parameters of the metabolic syndrome. We performed a systematic investigation of APM1 and its role in modulating adiponectin plasma concentrations in a particularly healthy population of 1727 well-phenotyped Caucasians. Genotyping 53 SNPs (average spacing of 0.7 kb) in the APM1 gene region in 81 Caucasians revealed a two-block LD structure and enabled comprehensive tagSNP selection. We found particularly strong association with circulating adiponectin for 11 out of the 18 tagSNPs genotyped subsequently in the 1727 subjects up to a difference of 3 g/ml adiponectin concentration (increased levels: 14811, p<0.000001; -11388 p<0.000001; 45, p=0.0006; 276, p=0.00007; 712, p<0.000001; Y111H, p=0.04; 3639, p=0.001; 4545, p=0.01; decreased levels: 10066, p<0.000001; 8564, p=0.00002; and -450, p=0.0003). Haplotype analysis provided a thorough differentiation of adiponectin levels with 9 out of 17 showing significant association (5 with p-values<0.0001) also supported by a minimal spanning net. No significant association was found for any of the parameters of the metabolic syndrome. We thus present the first study based on fine-mapped APM1 SNPs in combination with a haplotype tagging SNP approach showing strong associations of APM1 variants with adiponectin concentrations in healthy subjects. The revealed two-block structure may point towards two causal regions, one including the promoter SNPs and a second the relevant exons. Our data on healthy subjects further suggests that modulation of adiponectin concentrations by variants in APM1 is not merely a concomitant effect in the course of disease but most apparent already in a healthy status.

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Comparison of direct versus indirect measures of insulin resistance in genetic studies using a novel compartmental modeling-based approach. *R.M. Watanabe, Z. Guan, D.V. Conti.* Dept Preventive Medicine, Keck School of Medicine at USC, Los Angeles, CA.

Insulin resistance (IR) is a hallmark of type 2 diabetes. Direct assessment of IR can be impractical for large genetic studies, resulting in use of easily obtained indirect indices of IR based on fasting concentrations or oral glucose tolerance test (OGTT). Indirect indices correlate with direct IR measures, but data suggests the correlation is largely due to environmental, rather than genetic factors. The aim of this study is to use computer simulation to compare the ability of direct vs. indirect IR measures to accurately detect and characterize genetic variation underlying IR. We developed a compartmental model-based system that simulates the effect of genetic variation on glucoregulation. The model is physiologically-based, takes into account the feedback regulation between glucose and insulin, and is capable of simulating clinical research protocols, e.g., OGTT and intravenous glucose tolerance test (IVGTT). Genetic variation is introduced by assigning genes to specific model parameters to simulate specific genetic defects. For each gene, genotype specific parameter values are derived from a population distribution, constrained by gene frequencies and assuming an additive genetic model. A sample of OGTTs/IVGTTs are simulated assuming one gene affecting IR and another gene affecting -cell function. A common indirect index of IR, Stumvoll index (St_I), is computed from the simulated OGTT. Simulated IVGTT data are analyzed by minimal model to derive the insulin sensitivity index (S_I), a direct measure of IR. St_I and S_I are used as phenotypes to test for association with the two genes introduced in the simulation. Initial results show both St_I and S_I have good power (47% and 63%, respectively) to detect genetic variation underlying IR. However, St_I lacks IR specificity and detects variation underlying -cell function (41%), while S_I does not (17%). Additional simulations are under way. We conclude that while both indices allow detection of genetic variation underlying IR, St_I also incorrectly detects association with genes underlying -cell function.

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Insulin VNTR is a determinant of over-mortality associated with severe obesity. *D. Meyre[1], F. Degraeve[1], B. Heude[2], V. Vatin[1], M. Ghoussaini[1], E. Durand[1], P. Boutin[1], C. Dina[1], M.A. Charles[2], P. Froguel[1, 3].* 1) CNRS UMR8090, Lille, France; 2) INSERM U258, Villejuif, France; 3) Imperial College, London, UK.

We analyzed INS VNTR-linked -23 Hph1 polymorphism in 2 French family samples: 286 population-based pedigrees (484 children, 434 parents) and 449 selected pedigrees (633 obese children, 804 parents and 517 grandparents). We observed a borderline over transmission of the VNTR class I allele (transmission rate: 55%, p=0.07) from parents to children in the whole population-based cohort, whereas the class I allele was associated with childhood overweight (p=0.0005) and childhood obesity (p=0.009). In the obese children cohort, class I allele was associated with higher % of fat mass (p=0.04), higher insulin secretion (p=0.004) and higher % of binge-eating (p=0.05). In parents from the general population, class I allele was associated with severe obesity (p=0.007) and glucose intolerance/type 2 diabetes (p=0.05). In parents from childhood obesity families, class I allele was associated with morbid obesity (p=0.05), higher food disinhibition score (p=0.07), lower HDL cholesterol/total cholesterol ratio (HDL/TOT) (p=0.06) and higher triglycerides level (TG) (p=0.03). In grandparents, class I allele was associated with obesity (p=0.02), lower HDL/TOT (p=0.005) and higher TG (p=0.03). Morbid obesity prevalence was 2-fold higher in obese children parents (9.5%) compared to their grandparent (4.4%, p=0.0005). When comparing genotype distribution between the 3 generations in childhood obesity pedigrees, we observed an over-representation of III/III genotypes in grandparents in comparison with parents and obese children (OR=1.45 and OR=1.58, p=0.06 and p=0.06) inducing modest Hardy-Weinberg disequilibrium (p=0.01). Our results suggest that the VNTR class I allele 1) promotes better survival in the first stages of life (as suggested by its over transmission in children) 2) leads to a chronic severe obesity phenotype later in life through enhanced insulin secretion and possibly abnormal food intake 3) leads to reduced life span probably due to metabolic complications associated with severe chronic obesity. We propose that the INS VNTR could be a key determinant of the thrifty genotype.

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Demographic factors associated with participation in genetic studies as part of the National Birth Defects Prevention Study. *K. Crider[1], A. Woomert[2], J. Reefhuis[1], M. Honein[1].* 1) NCBDDD, CDC, Atlanta, GA; 2) Battelle, Durham, NC.

The National Birth Defects Prevention Study is a multicenter case-control study designed to examine both environmental and genetic risk factors for birth defects. We assessed demographic characteristics associated with completion of a mailed buccal cell collection kit after a telephone interview about prenatal exposures. The analysis was limited to participants from Atlanta with an estimated delivery date on or before 12/31/2001 who completed an interview (interview participation rate=74%). A total of 1,222 interviewed mothers were eligible to receive a buccal kit (946 case mothers, 276 control mothers). In this group 47% (n=572) were non-Hispanic White (White), 34% (n=413) were non-Hispanic Black (Black), 14% (n=165) were Hispanic, and 6% (n=72) were other or missing race information. The buccal kit was completed by 46% of the interviewed mothers and participation rates were highest among Whites (60%), followed by other (40%), Hispanics (38%) and Blacks (30%). In a multiple logistic regression model, Black race (OR 0.3; CI 0.2-0.4) and Spanish interview (OR 0.3; CI 0.2-0.5) were associated with decreased participation, while wanting to become pregnant at the time of conception (OR 1.5; CI 1.1-1.9) was associated with increased participation. Among White mothers, over 12 years education (OR 2.1; CI 1.1-2.5), wanting to become pregnant at the time of conception (OR 1.7; CI 1.1-2.5) and having a child with a birth defect (OR 1.7; CI 1.1-2.7) were associated with increased participation. Among Black mothers, over 12 years education (OR 0.58; CI 0.3-0.95) and household income under $20K a year (OR 0.57; CI 0.34-0.96) were associated with decreased participation. Among Hispanic mothers, Spanish interview (OR 0.3; CI 0.1-0.5) was associated with decreased participation, while folic acid or multivitamin use before pregnancy (OR 2.53; CI 1.17-5.45) increased participation. Although women were willing to participate in an extensive interview, low participation in the genetic component limits both the power for analysis and the generalizability of results. Evaluations and interventions to increase participation might need to be tailored to individual groups.

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Evidence of gene-gene interaction of DRD4 and DAT1 loci for increased susceptibility to attention-deficit/hyperactivity disorder in Chilean families. *X. Carrasco[1], P. Rothhammer[2], M. Moraga[2], H. Henriquez[2], F. Aboitiz[2], F. Rothhammer[2], R. Chakraborty[3].* 1) Hospital Calvo Mackenna, Santiago, Chile; 2) Human Genetics Program, Faculty of Medicine, University of Chile, Santiago, Chile; 3) Center for Genome Information, Dept. Environmental Health, University of Cincinnati, Cincinnati, OH 45267.

Attention-deficit/hyperactivity disorder, ADHD (MIM 126452), is a common, highly heritable neurological disorder of childhood onset, characterized by hyperactivity, impulsiveness, and/or inattentiveness. We carried out a family-based discordant sib-pair analysis (with 26 cases and 25 controls) to detect possible associations between dopamine receptor D4 (DRD4) and dopamine transporter 1 (DAT1) polymorphisms and ADHD in Chilean families. Classified as homozygote or heterozygote for the DRD4 7 repeat and DAT1 10 repeat alleles, both loci, individually did not exhibit genotype frequency differences between affected children and their healthy siblings (Fishers exact test $p > 0.25$ in both cases). However, simultaneous presence of DRD4 7 repeat heterozygosity and DAT1 10 allele homozygosity was significantly higher (34.6%) in cases, compared with their unaffected siblings (4%; Fishers exact test $p = 0.0096$; odds-ratio, OR = 12.71). Increased density of dopamine transporter in ADHD brains, along with abundance of 7 repeat D4 receptors in prefrontal cortex, which is impaired in ADHD patients, make the observed gene-gene interaction worthy of further studies. (Data analysis of this research is supported by NIH grant GM 41399 to RC).

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Hereditary hemochromatosis of type I: influence of body mass index on biological expressivity. *V. Scotet[1], G. Le Gac[2], F. Boisbras[1], C. Ka[2], MC. Mérour[2], C. Férec[1,2].* 1) INSERM U613, Brest, France; 2) Etablissement Français du Sang - Bretagne, Site de Brest, France.

Hereditary hemochromatosis is a disorder of iron metabolism, whose main form is usually associated with missense mutations in the HFE gene. The phenotypic expression of the most common HFE genotype (C282Y/C282Y) is heterogeneous and its expressivity is influenced by environmental and genetic factors. The aim of this study was to assess the influence of body mass index (BMI) on the disease expressivity in a cohort of patients homozygous C282Y/C282Y. The study included the C282Y homozygous patients enrolled in a phlebotomy program at the blood centre of Brest (western Brittany, France). A clinical questionnaire was completed at the time of diagnosis. The body mass index (BMI) was assessed and divided in three classes (normal: BMI25 kg/m², overweight: BMI25 and 30, obesity: BMI30). A multiple logistic regression was used to determine the effect of BMI (coded as two dummy variables) on elevated serum ferritin (cut-off of 1000 g/L), after adjustment on potential confounders. This study included 381 patients, among whom 51.2% were males (n=195). The BMI was documented for 346 patients (90.8%). Among them, 18 were obese (5.2% i.e. 3.4% of males and 6.9% of females) and 104 overweight (30.6% i.e. 36.6% of males and 23.6% of females). Serum iron parameters were significantly higher in the group of obese and of overweight patients than in the other group (p0.001). The risk for patients with overweight to have a ferritin level greater than 1000 g/L was 1.7 times higher than in patients with normal BMI (OR=1.7 [1.0; 3.0], p=0.043). This risk was 3.2 times higher for obese patients (OR=3.2 [1.2; 8.8], p=0.019). After adjustment on potential confounders such as gender, age at diagnosis and alcohol consumption, only the risk associated with obese patients remained significant. This study highlights the influence of BMI on the biological expression of hereditary hemochromatosis. This contributes to explain a part of the heterogeneity observed in the phenotypic expression of the C282Y/C282Y genotype.

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Meta-analysis of genome-wide linkage studies for quantitative lipid traits in African Americans. *A. Malhotra[1], H. Coon[2], M.F. Feitosa[3], W. Li[4], K.E. North[5], R.A. Price[4], C. Bouchard[6], S.C. Hunt[7], J.K. Wolford[1].* 1) Diabetes & Obesity Unit, TGen, Phoenix, AZ; 2) Psychiatry Dept, U of Utah, Salt Lake City, UT; 3) Div of Biostatistics, Washington U, St. Louis, MO; 4) Dept of Psychiatry, U of Pennsylvania, Philadelphia, PA; 5) Dept of Epidemiology, U of North Carolina, Chapel Hill, NC; 6) Pennington Biomedical Center, Louisiana State U, Baton Rouge, LA; 7) Cardiovascular Genetics Div, U of Utah, Salt Lake City, UT.

To date, five genome scans, including two independent analyses using the same population, have been performed to identify loci underlying quantitative lipid levels in African Americans, but with results that have been largely inconclusive. Linkage analyses are often limited by both sample size and heterogeneity, which may lead to nominal LOD scores or lack of evidence for linkage; the use of meta-analysis to combine linkage results from populations with similar ethnic backgrounds has been proposed as a method to overcome these issues. Thus, we performed a meta-analysis using data from lipid studies conducted in African American families to identify genetic regions showing evidence for linkage across studies. The genome scans were performed using data from: 1) the HyperGen study, 2) obesity-ascertained families, 3) the GENNID study, and 4) the HERITAGE study. We used the Genome Scan Meta-Analysis program to assess evidence for linkage for the following traits: total cholesterol, triglycerides (TG), LDL-C, and HDL-C. In the meta-analysis, p-values were estimated when combining the linkage results for a given lipid trait and were weighted by study size. In addition, a Bonferroni correction was applied to assess genome-wide significance. Significant evidence (i.e., $p<0.00042$) for linkage was found for LDL-C on chromosome 1q32.1-q41 ($p=0.00014$) and 1q41-q44 ($p=0.00017$). We also found suggestive evidence (i.e., $p<0.00847$) for TG on 16p12.1-q11.2 and for HDL-C on 4p15.1-p11. These results indicate that meta-analysis can be a valuable tool for increasing power to detect linkage. Importantly, statistically significant evidence for linkage on chromosome 1q suggests that this region may harbor a gene underlying the inheritance of LDL-C in African Americans.

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Identification of two regions predisposing to HTLV-1 infection in childhood by a model-based genome-wide linkage analysis. *S. Plancoulaine[1], A. Gessain[2], P. Tortevoye[2], A. Boland[3], L. Abel[1].* 1) INSERM U550 - HGID, Necker Medical Sch, Paris, France; 2) EPVO, Pasteur Institute, Paris, France; 3) Centre National de Genotypage, Evry, France.

Background Human T-cell leukemia/lymphoma virus type 1 (HTLV-1), is a human oncoretrovirus which causes an adult T-cell leukemia/lymphoma and a chronic neuromyelopathy. We previously showed by segregation analysis that a major gene controlled HTLV-1 infection in children of African origin living in a high endemic area. The aim of the present study was to map this major gene by means of a genome-wide scan.

Methods A total of 52 subjects belonging to 5 families were genotyped for 382 microsatellites markers spanning the entire genome. Linkage analysis was performed using a dominant model of transmission with parameters estimated by our previous segregation analysis. We conducted two kind of analyses. The first one, denoted as affected only, considered only the HTLV-1 seropositive subjects. In addition to seropositive subjects, the second strategy, denoted as intermediate, also considered seronegative offsprings born of seropositive mothers. Refined mapping was performed within regions with at least suggestive evidence for linkage (lod score = 2).

Results Two regions provided evidence for linkage. The first region was identified on chromosome 2 (lod score = 2.94) using the intermediate analysis. Evidence for linkage to this region was even stronger (lod score = 3.04) when considering only the larger pedigree of the sample. The second region was identifed on chromosome 6 (lod score = 2.89 in the whole sample) by the affected only analysis. The investigation of one candidate gene within each region is ongoing.

Conclusions Genome-wide linkage studies confirm the existence of at least one major locus controlling susceptibility to HTLV-1 infection in children (i.e. acquired through breast-feeding) of African origin.

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Asso-Link : association with the evidence of linkage when parents are not typed. *C. DINA[1], C. LECOEUR[1], P. FROGUEL[1,2].* 1) Lille Biology Institute , CNRS, Lille, Nord-PdC, France; 2) Genomic Medicine and Genome Center, Hammersmith Campus, Imperial College London, UK.

When genetic linkage has been identified, association analysis of single-nucleotide polymorphisms (SNPs) is carried out within the linkage region. If a SNP shows evidence of association, it is useful to know whether it can explain the linkage result. We have developed a method, Asso-Link, that evaluates the lod-scores for affected sib-pairs Identical By State (Boutin, et al., 2003). We here evaluate the properties of our method when the parents are not typed, which is common for complex diseases and propose an extension to general pedigree structure and quantitative traits. Asso-Link evaluates the relation of the candidate SNP genotype vector (Gj) with the linkage statistic. This method uses all the possible inheritance vectors Sj, for a family, together with all the possible Founder Genotypes Labels (FGL) combinations, which defines the distribution of all Gi. The probabilities of the different FGL states, when the parents are untyped, depends on allele frequencies (F) and non-founders genotypes (NFG). The relation between the lod-score and Gj is compared to its expected value under several H0 hypotheses. To investigate the methods properties, we simulated familial data under a model including single gene, polygenic and environmental effects. From simulated data, we extracted families with homozyguous parents to estimate the confounding between association with trait and association with the linkage. Our method yields correct p-values under the hypothesis of no association, with 5 percent of replicates displaying a nominal p-value of 0.05. However, when parents are not genotyped, the choice of F influences the p-value for association with linkage. When we use the population allele frequency, our Asso-Link test is a simple test of association whereas using the allele frequency estimated in the sample and the NFG data assesses the real impact on linkage. Although a significant association result is important, we need to distinguish this result with the association with the evidence of linkage. In terms of power, our method performs as well as similar tests like GIST.

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Indication for a pleiotropic effect of a quantitative trait locus (QTL)on two asthma-related phenotypes in 295 French EGEA families. *A. Ulgen[1], E. Bouzigon[1], M.H. Dizier[2], J. Maccario[3], C. Krähenbühl[1], A. Lemainque[4], M.P. Oryszczyn[3], F. Kauffmann[3], M. Lathrop[4], F. Demenais[1].* 1) INSERM EMI0006, Evry; 2) INSERM U535, Villejuif; 3) INSERM U472, Villejuif; 4) Centre National de Génotypage, Evry, France.

A genome-wide screen for asthma and seven asthma-related phenotypes was recently conducted in 295 EGEA families ascertained through asthmatic probands. This scan showed potential evidence for linkage of 21q22 region to two asthma-related phenotypes: %predicted FEV1 (forced expiratory volume in 1 second, a quantitative measure of lung function) and SPTQ (number of positive skin test responses to 11 allergens, a measure of allergen polysensitization). Moreover, principal components of genome-wide lod scores indicated that these phenotypes may share genetic determinants. To investigate whether the 21q22 region may contain a QTL with a pleiotropic effect on %FEV1 and SPTQ, we conducted a multivariate linkage analysis based on Haseman-Elston regression method, using a fine map of 13 microsatellites (average spacing of 3 cM). This multivariate analysis indicated linkage in the same region as univariate analyses with a p-value of 0.0001 (at 35 cM), this p-value being lower than the p-values previously obtained when analyzing %FEV1 and SPTQ separately (p=0.002 for %FEV1 and p=0.004 for SPTQ). This result suggests that the 21q22 region may contain a QTL underlying these two phenotypes. Further analysis based on bivariate variance component method will be carried out to further investigate this finding.

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Comorbidities in ADHD: Pleiotropy and New Susceptibility Loci. *M. Jain[1], L.G. Palacio[1], F.X. Castellanos[2], J.D. Palacio[3], D. Pineda[3], M.I. Restrepo[3], J.F. Muñoz[3], F. Lopera[3], D. Wallis[1], K. Berg[1], J.E. Baily-Wilson[1], M. Arcos-Burgos[1], M. Muenke[1].* 1) Natl Human Genome Research, Natl Inst of Health, Bethesda, MD; 2) New York University Child study Center, New York, NY, USA; 3) Neurosciences Group, University of Antioquia, Medellin, Colombia.

Attention deficit/hyperactivity disorder (ADHD) that is comorbid with Oppositional Defiant Disorders (ODD), Conduct Disorder (CD) and Substance Abuse/Dependence appears to be a subset of the phenotypic ADHD spectrum. Because of its high heritability this combination of behavioral disorders has been particularly suited for molecular genetic studies. We applied segregation and linkage analyses in a set of extended and multigenerational families of a genetic isolate densely segregating ADHD comorbid with disruptive behaviors such as ODD, CD, Alcohol Abuse/Dependence, and Nicotine Dependence and found evidence for the presence of pleitropy. Our data suggest that ADHD plus comorbid disruptive behaviors are monogenic cosegregating as a discrete unit as evidenced by the highly significant linkage among these traits: ADHD and ODD (LOD = 14.19), ADHD and CD (LOD = 5.34), ODD and CD (LOD = 6.68) and CD and Alcohol Abuse/Dependence (LOD = 3.98). In addition to previously reported ADHD susceptibility loci, 4q13.2, 5q33.3, 8q11.23, 11q22, and 17p11, we found significant evidence of linkage for this severe ADHD phenotype to a locus at 8q24, and suggestive evidence of linkage for loci at 2p21-22.3, 5p13.1-p13.3, 12p11.23-13.3, 8q15, and 14q21.1-21.2, 1. These results were replicated using as affected status a phenotype constituted by latent classes clustering individuals with ADHD plus disruptive behaviors symptoms. Based on our data we conclude that patterns of cosegregation of ADHD with comorbidities are crucial for understanding the inheritance patterns not only for ADHD, but also of other behavioral disorders in general. Furthermore, a refined description of the severe ADHD phenotype involving the cosegregation profile of comorbidities will be a powerful tool to define significant regions of linkage in the human genome.

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Evaluating power and false positive rates using Gaw 14 data. *R. Li[1], Y. Yao[2].* 1) Life Science, Fudan University, shanghai, China; 2) Epidemiology, Johns Hopkins Bloomberg School of Public Health.

FBAT is a unified approach based on the classical TDT method. The basic idea underlying this method is to compute the distribution of FBAT statistic under the null hypothesis, conditional on the observed phenotypes, and compare the statistic distribution deriving from observed genotype to it. It allows the use of different genetic models and tests of different null hypotheses. The aim of our project is to investigate the effect of different options in FBAT on power and false positive rate. In order to test type-I error we used all purchased packets, and the ones that involving the chromosomes 1 and 3 where disease loci locate were excluded. And all the 100 replicates were analyzed. False positive rate (FPR) is defined as proportion of markers of which test statistic gets significant p-value (0.05). We performed multiple FBAT analyses to compare the FPR using different inheritance models, different sets of offset and different null hypothesis. We evaluated the power to detect association using the SNP marker B03T3067. It situated on top of the disease locus involved in P1. The power for association analysis is defined as the number of replicates gave a p-value less than 0.05. Power under different genetic model, different use of offset and different null hypothesis was calculated. We used Haploview to perform association analysis on marker B03T3067 for the purpose of comparison. Under dominant model the false positive rate is 0.09, which is much higher than empirical type-I error. To calculate power, we performed FBAT analysis using B03T3067. It was carried out under different model of inheritance, diffent offsets. Power under dominant model is higher than additive one if we chose to use a general FBAT statistic. However there is insignificant difference between two models if we used the -e option. For incorporating offset in construction of test statistic -o option did not improve power. In conclusion, we found that false positive rate is inflated under a dominant model.

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Comparisons of mining methods for gene mapping using family-based and population-based SNP data. *C.H. Chen[1], L.Y. Wei[1, 2], C.L. Huang[2, 3].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Information Management, Huafan University, Taipei, Taiwan; 3) Department of Information Management, National Kaohsiung First University of Science and Technology, Kaohsiung, Taiwan.

Data mining methods have been widely applied to deal with complicated data. Several studies had demonstrated the use of data miming skills in genetic research. However, some of the methods were developed under specific genetic models or for data with restricted properties. Improper use of these methods might result in false positives. This study aims to symmetrically assess the application of four data mining methods for detecting genes susceptible to diseases. Family-based and population-based data are simulated under a grid of genetic models. Genetic factors considered in the disease locus are inheritance mode, disease allele frequency, and penetrances. Two-locus Heterogeneity is also incorporated in the data. Qualitative traits are generated based on disease locus. Linked SNP markers in candidate regions are simulated with various recombination fractions and first order linkage disequilibrium measurements. In this study, four data mining methods are inspected, including Decision Trees (C4.5 and RTREE), Johnson Algorithms combined with Rough Set Theory, and Genetic Algorithms combined with Rough Set Theory. Power or the probability of correctly identifying the susceptibility genes is calculated for comparison. Our results show that, in general, high penetrances lead to higher powers under all models for all methods. However, the four methods fail when the perentrance is lower than 0.25. The combining of genetic algorithms and Rough set theory produces the highest power in family-based data under single gene models, while, under two-locus heterogeneity models, Decision trees C.5 and RTREE has the highest power in population- and family-based data respectively. The four methods have similar performance in population-based data under single gene models. In summary, the use of data mining methods should base on the characters of data in order to reduce false positives.

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Using conditional linkage to detect epistasis or heterogeneity. *D. Shmulewitz[1,2], J. Zhang[2], L.J. Strug[2], D.A. Greenberg[1,2,3].* 1) Department of Psychiatry; 2) Division of Statistical Genetics, Department of Biostatistics, Columbia University, New York, NY; 3) New York State Psychiatric Institute, New York, NY.

Introduction: Complex diseases are most likely caused by a number of genes. Each may act alone (heterogeneity, H), or interact (epistasis, E), or a combination of both. We are often able to identify such genes singly, using linkage analysis. But we need to determine if these genes act alone or interact to cause disease. One suggestion is to condition on the linkage at one locus to determine if a second locus is involved. In this scheme, to detect E, families with positive npl scores at the first locus are used to calculate an adjusted npl score for locus 2 (2-adj). To detect H, families with negative npl scores for the first locus are used to calculate 2-adj. Depending on the above weighting scheme, if 2-adj is greater than the original locus 2 score (2-orig), that is evidence for either E or H. **Method:** We tested this method by simulating 100 datasets of 100 families for a number of genetic models, with E, H, or both. By design, linkage existed and was detectable at the two loci being tested. E or H were detected if 2-adj was greater than 2-orig. **Results:** H was detectable for two genetic models, R-R (recessive at both loci) and R-D (recessive at locus 1, dominant at locus 2), but only when the original evidence for linkage was much greater at the first locus. The empirical power to detect H (% datasets with 2-adj > 2-orig) was 51.1% and 58.8% for the R-R/R-D models. E was detectable only for the RR (recessive at both loci) model, but only when data were simulated such that a third locus independently caused disease. The power to detect E was 61% when the third locus was recessive, and 52% when it was dominant. **Discussion:** Thus only in certain cases will conditioning on linkage at one locus indicate evidence for a more complex genetic model. Even in these experiments with ideal data the power is low. Therefore, conditioning on linkage at one locus may be of limited utility in determining the underlying genetic models.

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Random forest analysis: a novel approach for exploring the genetic component in multiple sclerosis (MS). *E. Madden[1], A. Cutler[2], S. Sawcer[3], A. Compston[3], J.R. Oksenberg[1], S.L. Hauser[1], M.A. Pericak-Vance[1], J.L. Haines[1], G. Stewart[4], L. Peltonen[5], L. Massacesi[6], T. Masterman[7], J. Hillert[7], M.G. Marrosu[8], M. Eraksoy[9], L.F. Barcellos[1].* 1) US MS Genetics Group, UCSF; 2) Utah State Univ; 3) Univ of Cambridge, UK; 4) Univ of Sydney, AU; 5) Univ of CA, Los Angeles; 6) Univ of Florence, IT; 7) Karolinska Inst, SE; 8) Univ of Cagliari, IT; 9) Univ of Istanbul, TR.

Despite strong evidence for polygenic inheritance in MS, only the MHC (ch. 6p21) region has consistently demonstrated linkage and association. It appears that the HLA-DRB1*15 association largely explains this linkage signal; however, the exact mechanism(s) by which MHC gene(s) operate in MS are not known. Genome screens have been performed in MS, but results suggest the existence of genes with strong individual effects in MS is not likely. Novel non-parametric approaches such as tree-based classification methods that accommodate large marker datasets and allow for genetic heterogeneity may be useful for MS studies. Recent theoretical work has also shown that the prediction accuracy of classification trees is significantly improved by building a collection of trees, or a so-called Random Forest(RF). Whole genome screen data for 824 MS ASPs from 8 populations was pooled and analyzed using a RF approach, to identify evidence for loci potentially interacting with the MHC region. Genetic sharing (mean IBD) at other regions of the genome (3 cM map intervals), country and gender distribution for each MS sib pair (total of 1176 predictors) were used to predict sharing at HLA-DRB1 (outcome). The no. of variables considered at each node was 400; the final error rate was 2.3 % (based on 10,000 trees). As expected, the genomic regions where sharing was most important in predicting DRB1 sharing were in close proximity to this locus (+/-3 cM on each side); however, no evidence for country, gender or sharing at loci outside of the MHC as predictors of DRB1 sharing was observed. Using this novel approach, our results strongly suggest that loci outside the MHC are not contributing substantially to influence risk in patients stratified by sharing at DRB1.

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Recruitment strategies and DNA marker selection for maximizing power of linkage analysis. *A.L. Maes[1,2], S.R. Diehl[2].* 1) Merck Research Laboratories, Rahway, NJ; 2) Center for Pharmacogenomics & Complex Disease Research, New Jersey Dental School, UMDNJ, Newark, NJ.

A common ascertainment strategy is the affected sibpair design. Previous studies suggest that recruitment of parents of affected sibs is highly desirable, but this is difficult for diseases with late age of onset. DNA may be unavailable from deceased parents, and its often not possible to confidently assign disease phenotypes for parents. Less attention has been paid to unaffected sibs of affected sib pairs. We used computer simulation to evaluate the relative contributions to linkage mapping power of parental disease phenotypes and availability of marker genotypes for parents and/or for 1 to 3 unaffected sibs. Penetrance of 25%, 50% and 75% under dominant and recessive transmission were evaluated. Several recent studies have examined the power of high density SNP maps as alternatives to microsatellites. We simulated a high density SNP map with markers spaced at 0.1 cM intervals and a map of SNP islands (3 SNPs located 0.1 cM apart with islands at 2 cM intervals). We compared these to microsatellites (8 alleles) spaced at 2-, 5- and 10-cM distances. Lastly, we introduced genotype errors of 1% or 5% to compare loss of power for our various pedigree structures, genetic maps, and disease models. Simulated data were analyzed using GENEHUNTER (GH, v2.1r5). HLOD scores under the true (generating) model and maximized over disease models with correction (MMLS-C), and the Information Content (IC) measure were calculated by GH. Results demonstrate that i.) parental disease phenotypes increase power more than parental marker genotypes; ii.) adding a single unaffected sib often adds as much or more power as both parents, even at low penetrance; iii.) SNP islands perform substantially better than 10 cM microsatellite maps, equivalent to 2 cM microsatellite maps or SNPs at 0.1 cM intervals; iv.) 1% SNP genotyping error causes > 50% loss of power in data sets of affected sibs only, but even 5% error is compensated by genotyping either both parents or 2 unaffected sibs (deleting markers in families with inconsistencies); the IC statistic fails to reflect these differences in power.

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Gene dropping vs. empirical variance estimation: A comparative study of standardization methods for allele-sharing statistics. *J. Jung, D.E. Weeks, E. Feingold.* Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

In this study, we compare the statistical properties of a number of methods to find p-values for allele-sharing statistics in nonparametric linkage analysis. Some of the methods are based on the normality assumption, using different variance estimation methods, and others use simulation (gene-dropping) to find empirical distributions of the test statistics. For variance estimation methods, we consider the perfect variance approximation and two empirical variance estimates. The simulation-based methods are gene-dropping with and without conditioning on the observed founder alleles. We also consider a Monte Carlo method proposed by Lin and Zou (2005). We discuss the analytical properties of these various p-value estimation methods, and then present simulation results comparing them. Assuming that the sample sizes are large enough to justify a normality assumption for the linkage statistic, the best p-value estimation method depends to some extent on the (unknown) genetic model and on the types of pedigrees in the sample. If the sample sizes are not large enough to justify a normality assumption, then gene-dropping is the best choice. We discuss the differences between conditional and unconditional gene dropping.

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Importance of allowing for dominance in variance components linkage analysis: Examples using dyslexia and cardiovascular disease. *N.H. Chapman[1], R.P. Igo Jr.[1,2], E.M. Wijsman[1,2].* 1) Department of Medicine, Division of Medical Genetics; 2) Department of Biostatistics; University of Washington, Seattle, WA.

Variance component (VC) methods are a popular approach to linkage analysis, in part because they are generally believed to be model-free. The model implicitly assumed in most software includes an additive major gene, and a polygenic component in which the individual loci are all additive. In fact, any locus may act in a way that is not exactly additive; in this case a non-zero dominance VC exists. If only sib-pairs are available, the additive and dominance VCs cannot be separately estimated. However, when extended families are available, dominance VCs can be estimated.

Models including dominance VCs can be fit using Genehunter (for small pedigrees) and SOLAR. In recent work involving a quantitative trait related to dyslexia in non-randomly ascertained families, we used a model that included a dominance VC for the major gene, but not in the polygenic part of the model. The results were difficult to interpret, due to a high background lod score in regions of the genome where no signal was apparent. Including a dominance VC in the polygenic part of the model solved this problem, demonstrating the importance of including dominance VCs in both parts of the model.

The question remains as to whether including dominance VCs will yield results that are qualitatively different from using a model with additive variance alone. The answer is dependent on the data set and trait in question. We give an example of HDL-cholesterol levels in 4 pedigrees of between 48 and 88 individuals where the inclusion of dominance VCs results in a substantial increase in the lod score (from 0.4 to 1.7). These pedigrees are extended families with multiple large sibships, which is exactly the situation where one would expect to get good estimates of both additive and dominance variance components.

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Analysis of continuous covariates in affected sib-pair linkage studies: Am I finding susceptibility or quantitative trait loci? *S. Schmidt, M. Schmidt, E.R. Martin, E.R. Hauser.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Data sets ascertained for the genetic analysis of complex human diseases often include a number of continuous covariates, which may be risk factors for a dichotomous disease phenotype, endophenotypes, quantitative biomarkers, or other disease-related phenotypic features. These covariates may influence the disease trait in two different ways: (A) They may be truly environmental, but may define a more genetically homogeneous subset of families with increased linkage evidence for a disease susceptibility locus, compared to the entire data set. (B) They may themselves have a genetic basis, causing the underlying quantitative trait locus (QTL) to contribute indirectly to disease risk. We performed a simulation study with the software SIMLA in order to evaluate whether the application of two distinct analysis methods to the same data set, the ordered subsets analysis (OSA) and a QTL analysis (MERLIN-REGRESS), would be informative for distinguishing between (A) and (B). Our results can be summarized as follows: (i) When the covariate is a risk factor for disease and its distribution in the population is influenced by a QTL, both OSA and MERLIN-REGRESS are very likely to detect the QTL. (ii) When the covariate is not a risk factor but does have a genetic basis, OSA is much less likely than MERLIN-REGRESS to detect the QTL. (iii) When the covariate distribution is not influenced by a QTL but confers a greatly increased disease risk only in the presence of a disease susceptibility genotype (gene-environment interaction), OSA is much more likely than MERLIN-REGRESS to detect linkage to the disease susceptibility locus. These findings assist in the interpretation of analysis results in real data sets. They suggest that concordance or discordance of OSA and QTL results may be indicative of underlying genetic mechanisms related to disease risk.

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Comparison of two binning strategies for linkage genotypes on blood and archival tissue samples: the PACGENE experience. *G. Petersen[1], S. Grimes[2], K. Rabe[2], C. Olswold[2], M. Goggins[3], M. de Andrade[2].* 1) Health Sci Research, Mayo Clinic, Rochester, MN; 2) Biostatistics, Mayo Clinic College of Medicine, Rochester, MN; 3) Johns Hopkins University.

The Pancreatic Cancer Genetic Epidemiology (PACGENE) consortium comprises seven institutions with ongoing efforts to rapidly ascertain and recruit familial pancreatic cancer patients and relatives, including accrual of biospecimens, tumor tissue, and risk factor data, with the goal of susceptibility gene discovery by linkage analysis. To date, 50 families with 412 blood samples and 31 archival tissues have been genotyped for 405 microsatellite markers at the Center for Inherited Disease Research (CIDR). Feasibility of genotyping DNA from archival tissues and combining these data with blood DNA genotypes on pedigrees from the Center for Inherited Disease Research has been demonstrated by the Genetic Epidemiology of Lung Cancer Consortium. However, our study is the first to use the same platform to generate genotypes from both lymphocytes DNA and DNA from archival tissues. We have developed an allele calling algorithm that accommodates the two sources of genotypes. To assess the efficiency of our algorithm, we compared it to the conventional CIDR allele binning algorithm. . Concordance rates on alleles called by the two algorithms were performed for all autosomal chromosomes. The average rate was 88%, with minimum and maximum rates of 66.7% and 100% in 2 and 3 chromosomes, respectively. To evaluate the efficiency of these two strategies, we compared the Mendelian errors in these chromosomes. These preliminary results suggest the important role played by the type of markers and the length repeats in defining the allele calls.

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A novel method for linkage disequilibrium mapping of quantitative trait loci. *H.H.H. Goring[1], S. Nair[1], D. Sudimack[1], F. Nosten[2], J.T. Williams[1], T.J.C. Anderson[1].* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Shoklo Malaria Research Unit, Maesot, Thailand.

Some investigators view genome-wide linkage disequilibrium (LD) analysis as the most promising strategy for deciphering the genetic etiologies of complex traits in humans. This belief is spurred by vast improvements in genotyping technology and by the potential existence of haplotype blocks. Most applied genome-wide association studies, as well as related research on statistical methodology, have focused on dichotomous disease phenotypes in a case-control study design framework. The emphasis on clinical disease endpoints may be suboptimal, and quantitative endophenotypes correlated with disease may be more suitable targets---for linkage analysis and for LD analysis alike---because of their likely simpler etiology and larger information content. When multi-allelic data are used (such as microsatellites or SNP haplotypes), the power of association analysis may be greatly reduced because of the multitude of degrees of freedom (df). We have therefore adapted the approach developed by Terwilliger for LD analysis of multi-allelic data and discrete traits (AJHG 56:777) for use with quantitative traits. The key benefit of our approach is the reduction in number of df to 1, rather than the usual n - 1 (with n being the number of observed alleles or haplotypes). The reduction in df is achieved by making the critical assumption of a single ancestral functional site. The approach does not assume knowledge of which marker allele/haplotype is on the ancestral chromosome, and instead weights over all possibilities. The approach can be powerful even if the assumption of a single origin is not correct. We have applied our method by performing genome-wide association analysis for drug resistance loci in the genome of the malaria parasite *Plasmodium falciparum*. Using the approach, we were able to successfully detect association between mefloquine resistance and a known multi-drug resistance locus on chromosome 5 and have located a number of other potentially interesting candidate regions. Details of the statistical methodology and its application will be presented.

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Bias-Reduced QTL Effect Size Estimation Via Statistical Resampling. *L. Wu[1], L. Sun[2,3], S.B. Bull[1,2].* 1) Samuel Lunenfeld Research Institute,Toronto,Canada; 2) Department of Public Health Sciences, University of Toronto, Toronto,Canada; 3) Hospital for Sick Children, Toronto, Canada.

In genome-wide linkage analysis the locus-specific effect estimate is subject to upward selection bias because of the genome-wide maximization of the test statistic and the adoption of stringent significance criteria. Göring et al. (2001)demonstrated inflation in genetic effect estimation in a variance-components linkage analysis of quantitative-trait-loci (QTL). We employed Görrings simulation scheme and investigated the resampling techniques proposed by Sun and Bull (2005) to reduce the selection bias.We performed the linkage scan and locus-specific QTL-heritability estimation with an extension of Haseman-Elston regression procedure (Sham et al., 2002) implemented in Merlin (Abecasis et al. 2002). We constructed three bootstrap-based estimators: the shrinkage estimator, the out-of-sample estimator and the weighted estimator. We investigated each of six simulation settings (0 to 5 QTLs) with true locus-specific QTL-heritability of 0.1. In each of 500 replications we estimated the QTL-heritability at the locus with the maximum test statistic in the genome scan. All three estimators effectively reduced the upward bias in estimation of locus-specific QTL-heritability and the shrinkage estimator provided the best bias reduction overall. We also extended this method to jointly estimate the genetic effects of multiple loci for the case where there are several loci meeting genome-wide significance.

QTLs	True and False peaks		True Peaks		False Peaks	
	Naive	Shrinkage	Naive	Shrinkage	Naive	Shrinkage
1	.226	.035	.143	-.044	.242	.050
5	.250	.005	.211	-.033	.303	.058

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Mixed effects logistic approach for association following linkage scan for complex disorders. *H. Xu, S. Shete.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

Large scale population-based association studies at genomic level hold substantial promises for unravelling the genetic basis of complex disorders, in which the etiology usually involves both genetic and environmental factors. Family-based association procedures such as transmission disequilibrium test (TDT) has less power in detecting association than the properly controlled population-based procedures. Because of the relatedness of family members, using affected family members and unrelated normal control directly leads to false positive results in association studies. In this study, we propose a new approach using mixed model logistic regression in which associations were performed using family members and unrelated controls. Thus, the information contained in family members can be fully explored while retaining the high power. To examine the properties of the new approach, we developed an efficient algorithm to simulate environmental risk factors and the genotypes at both the disease locus and a marker locus with/without linkage disequilibrium (LD) in families. Extensive simulation study showed that our approach can effectively control the Type-I error rate. Our approach is better than family based designs such as TDT because it allows the use of unrelated cases and/or controls and it uses all the affected members in the study for whom DNA is possibly already available. Our approach also allows the inclusion of covariates such as age and smoking status. Our method can be further extended to accommodate more complex pedigree structures.

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On the use of maximal segmental score to estimate regional specific p-value in gene mapping studies. *C.S.J. Fann[1,2], I.B. Lian[3], C.L. Kuo[2].* 1) Epidemiology & Public Health, Inst Biomed Sci, Acad Sinica, Taipei, Taiwan; 2) Institute of Public Health, Yang-Ming University, Taipei, Taiwan; 3) Department of Mathematics, National Chang-Hua University of Education, Chang-Hua, Taiwan.

Identifying the susceptible disease gene is an important issue in biomedical research. To do this, one of the popular tools is to use highly-dense SNP markers to perform large scale association tests. In these studies, multiple test correction for type I error is needed. Conventional methods, such as Bonferroni¡¦s method, are conservative with low statistical power. Previously, we proposed using the distribution of the ¡§longest significant run¡¨ to attain ¡§region-specific p-value¡¨, in which a sequence of p-values from single marker tests were firstly converted into binary indices of zero and one according to a preset cutoff (e.g. p = 0.05). In this project, to reduce the loss of information due to a simple cutoff, we extended the idea of the run of dichotomous index to a more flexible ¡§maximal segmental score¡¨ of polychotomous weight according to the range of p-values. A few weighting strategies were considered. Simulations were carried out to choose the best strategy according to the statistical power and type I error rate under various assumptions on the penetrance, sample size, and disease allele frequency. The results show that weighting strategy using the function of log10 scale of p-value performed best. We also applied the best strategy to three authentic datasets that were available in the public domain and were able to confirm the previous findings.

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Excess homozygosity of haplotypes in the presence of population substructure and genetic admixture. *R. Chakraborty, X. Sheng.* Ctr Genome Infor/Env Hlth, 108, Univ Cincinnati, Cincinnati, OH.

Linkage disequilibrium (LD) is generally inferred from deviations of haplotype frequencies from the product of respective allele frequencies. For a pair of biallelic markers, a single LD parameter explains the non-random association of alleles between loci. When one or both loci are multiallelic, the overall non-random association of alleles between loci is a composite function of several allele-specific disequilibria measures. Further, since the observed data generally are in the form of genotypes, and not haplotypes, LD estimation has also been attempted by computing the deviation of multilocus homozygosity from the product of homozygosity values at the respective individual loci (the resultant statistic is called excess homozygosity, or identity excess, IE). Though this formulation does not contrast frequencies of each individual genotype with their expectations under the assumption of independence, IE has some interesting population genetic properties and it is easily computed and directly estimated from observed data. Like LD, IE is also affected by population substructure and genetic admixture. We provide a quantitative evaluation of enhancement of IE by the effects of population substructure and genetic admixture. We show that in the presence of genetic admixture, enhancement of IE by the process of admixture provides provides a better power of conducting a trend analysis of monatomic decay of LD with recombination distance between loci. Consequently, the use of IE-based inference may add power of disease gene mapping by using admixture linkage disequilibrium (MALD) in populations of mixed ancestry where the ancestral populations differ by disease prevalence as well as allele frequencies at the marker loci. Availability of genome-wide MALD SNP markers and their genotype data from the different SNP databases provide opportunity for evaluate the increased power of IE in relation to the traditional LD measure to study variation of LD in admixed versus non-admixed populations.(Research supported by a NIH grant GM41399).

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Is localization for complex disease genes via linkage analysis really that bad? *C.W. Bartlett, V.J. Vieland.* Ctr Statistical Genetics Res, Univ Iowa, Iowa City, IA.

It is frequently said that linkage analysis cannot accurately localize genes for complex disorders, and that localization only becomes accurate when linkage evidence is very strong. We set out to test these assumptions, using as our measure of location the position of the maximum 2-pt linkage statistic on a simulated map (for full information and no errors, this is equivalent to localization based peak multipoint lod). We simulated a two-marker map, and varied (1) marker informativeness, (2) pedigree structure, (3) the position of the disease gene relative to each marker, (4) the degree of complexity in the generating trait model, and (5) the degree of misspecification in the analysis model. Our outcome measure was P[M0], the probability that the marker closest to the trait locus (M0) yields the largest statistic. We found that P[M0] appears to be largely independent of both the observed lod score and also the assumed mode of inheritance. Moreover, complicating the generating trait model by introducing locus heterogeneity actually increased the accuracy of localization. What does appear to drive P[M0] is the sample size N, independently of the ELOD associated with given N. E.g., under a simple recessive generating model, a recessive lod yields P[M0] = 52%-92% as N increases from 2 to 50 nuclear families; while analyzing the same pedigrees under a (wrong) dominant model yields P[M0] = 51% - 90% for the same values of N; but ELODs are essentially half as large under the dominant model at each value of N. Thus in these simulations, accurate localization does not depend on large lod scores, trait model misspecification does not adversely affect localization, and heterogeneity can actually improve localization - although *linkage detection* is affected by these things. Across all generating conditions considered thus far, at N = 50 nuclear families P[M0] ? 72.5%. In short, localization based on the 2-pt linkage peak appears to be reliable and robust. Current extensions of this work include consideration of sampling variability across a marker map and effects of marker density.

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Genome-wide variation in rates of gene conversion and crossover. *G. Hellenthal, M. Stephens.* Statistics, University of Washington, Seattle, WA.

Recombination is an important biological process that results in the "mixing-up" of human genetic material during cellular meiosis. There are two known kinds of recombination: crossover and gene conversion. There has been considerable recent progress in the development of statistical methods to infer fine-scale rates of crossover from population genetic data (i.e. genetic data on random samples of individuals from a population). Application of these methods to data from human populations suggests that average rates of crossover vary considerably among genes, and that within many genes a large proportion of crossover events occur within very restricted regions ("recombination hotspots"). In contrast, estimating rates of gene conversion has proved more difficult, and little is known about how such rates vary either among or within genes.

Here we aim to gain insight into the extent at which rates of gene conversion vary among genes. To do so, we have developed a statistical method (a modification of the "Product of Approximate Conditionals" (PAC) model of Li and Stephens (2003)) to jointly estimate rates of crossover and gene conversion. We use this within a hierarchical modeling framework to estimate the distribution among genes of the relative rate of gene conversion to crossover. By borrowing strength across genes, we attempt to estimate the parameters of this distribution, with an emphasis on estimating its variance. We applied this method to two datasets on human variation. Our studies thus far suggest that the relative rate of gene conversion to crossover, which researchers have previously assumed to be constant across the genome, may in fact vary by more than an order of magnitude.

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$_2$-Adrenergic Receptor Gene (*ADRB2*) Polymorphisms and Asthma in African Americans. *E.G. Burchard[1,2], N. Shaikh[1,2], H.J. Tsai[1,2], J. Kho[1,2], S. Choudhry[1,2], M. Naqvi[1,2], D. Navarro[1,2], H. Matallana[1,2], R. Castro[1,2], C. Lilly[3], H.G. Watson[4], K. Meade[5], M.L. Noir[6], S. Thyne[1], E. Ziv[1].* 1) University of California, San Francisco, CA; 2) Lung Biology Center, San Francisco General Hospital, SF, CA; 3) Brigham and Womens Hospital, Boston, MA; 4) The James A. Watson Wellness Center, Oakland, CA; 5) Children's Hospital and Research Institute, Oakland, CA; 6) Bay Area Pediatrics, Oakland, CA.

Ethnic-specific differences in genetic risk for diseases and drug response are well documented. $_2$-Adrenergic receptor gene (*ADRB2*) variants have been associated with asthma-related traits in different studies. However, association results have been inconsistent across various populations. Also, there is very limited information regarding genetic association between the *ADRB2* and asthma-related traits in African Americans. The biggest limitation for studying genetic association in African Americans is the confounding effect due to population stratification. We genotyped six known and recently identified SNPs, which were selected based on potential biological function and the haplotype structure. Thirty ancestry informative markers were genotyped and used to adjust the confounding effect due to population stratification in our analyses. We compared allele frequencies of the SNP +46 (*Gly^{16}Arg*) in our cohort (264 cases and 176 controls) with the ones reported in various populations including Caucasians, Chinese, Mexicans and Puerto Ricans. Significant genotype associations of bronchodilator response (FEF_{25-75} reversibility and FEV_1 /FVC reversibility) were found with the SNPs -468 and -47 (p=0.017 and 0.001 for FEF_{25-75} reversibility; p=0.037 and 0.012 for FEV_1 /FVC reversibility, separately). Significant haplotype association was shown between the haplotype combination (SNPs -47, +46 and 3'UTR) and asthma disease status. We did not observe association of asthma-related traits with the SNP +46 (*Gly^{16}Arg*), which has been associated in various populations. Our results underscore the importance of investigating genetic associations of the *ADRB2* and other asthma disease genes across different ethnic groups in asthma morbidity and bronchodilator drug responsiveness.

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Power Implications of the Dichotomized Continuous Trait FBAT. *D. Fardo, C. Lange.* Biostatistics, Harvard School of Public Health, Boston, MA.

Family-based association tests (FBATs) are used to test associations between a genetic marker and a disease susceptibility locus for many different scenarios. Here we examine the effects of using a binary affectation status based on a continuous phenotype. Based on simulation studies and analytical considerations, we propose a rule for this dichotomization that minimizes power loss under fairly general circumstances. The proposed approach is illustrated using a genetic asthma study.

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Linkage Analyses of Diabetic Nephropathy Phenotypes: The Family Investigation of Nephropathy and Diabetes (FIND). *S.R. Quade, K.A. Goddard, for the FIND Consortium.* Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH.

The FIND consortium was established to investigate genetic factors associated with diabetic nephropathy (DN) using both family-based and case control approaches. Genotyping was performed on 1227 individuals at 404 microsatellite markers from 378 families of African American (AA), American Indian (AI), European American (EA), and Mexican American (MA) descent. Model-free linkage analyses were conducted separately for each ethnicity using sib pairs concordant or discordant for DN, and p-values were pooled across ethnicities. We applied the Haseman-Elston (HE) procedure to quantitative intermediate phenotypes for DN including proteinuria and glomerular filtration rate (eGFR) estimated using the Modification of Diet in Renal Disease equation. Under the HE test we observed evidence of linkage for DN on chromosomes 7q, 10p and 15q (empirical p-values=5.99x10-4, 2.0x10-5 and 4.80x10-4, respectively). The AA families contributed to peaks on 7 at 102cM and 15 at 84 cM and the AI families contributed to the peak on 10 at 0 cM. We observed significantly decreased sharing among the discordant AA and AI sib pairs at locations on chromosomes 7 and 10 respectively, and slight evidence for decreased sharing on 15. Locations on 7 and 10 also show evidence of increased allele sharing among the AA and AI affected sib pairs respectively, consistent with the expected pattern of allele sharing for true susceptibility loci. Linkage signals for proteinuria were observed on 14q (94 cM, p=1.71x10-3) and 17q (82 cM, p=1.30x10-2) in MA families and 6q (80cM, p=2.65x10-3) in EA families, after adjusting for sex and age at diabetes onset. Suggestive linkage signals for eGFR were observed on 1q (266 cM, p=8.99x10-5) and 8p (52 cM, p=9.20x10-5) for the MA families, 6q (158 cM, p=4.48x10-4) and 15q (60cM, p=1.41x10-5) for the AI families, and 14q (55cM, p=2.69x10-3) for the EA families. In 2006, a complete genome scan will be performed using single nucleotide polymorphism markers. This scan will provide better power to detect our weaker signals and will hopefully replicate our significant signals.

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Study design and analysis issues in whole genome association studies. *J.C. Barrett[1], I. Pe'er[2], M.J. Daly[2], L. Cardon[1].* 1) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom; 2) Broad Institute of Harvard and MIT, Cambridge, MA.

Falling SNP genotype costs and the recent availability of genome-wide surveys of genetic variation have made whole genome association (WGA) studies imminent. Despite such advances these studies are still expensive and present significant analytical hurdles. It is important, therefore, to consider what the goal of a WGA study is, how well newly developed SNP panels address that goal and how downstream analysis choices influence the interplay of those two questions.

One crucial choice is deciding upon a set of "target variants" that we wish to test for association to disease. Alternative strategies have proposed focusing on coding variants, SNPs in genes or conserved regions, or all known variants. An orthogonal axis to this choice concerns the desired allele frequency spectrum. Further complicating these issues is the relationship between the set of genotyped SNPs and subsequent association tests and analysis.

Recent data from the HapMap project and Perlegen have made it possible to examine how well current and soon-to-be-available SNP genotyping products can capture "target sets", and to explore the consequences of initial marker selection on subsequent analyses. Using the available data, we compare different gene-based SNP panels with LD-derived and LD-agnostic panels of 100,000, 250,000 and 500,000 SNPs. We show how the initial genotyping strategy strongly influences later analysis choices and the eventual chances for identifying specific trait loci. Our results emphasize the importance of considering the entire study from SNP selection to final analysis in order to maximize power.

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Genotype-by-smoking interaction and burden of aortic atherosclerosis: The NHLBI Family Heart Study. *K.E. North[1], L.A. Lange[2], J.J. Carr[3], I.B. Borecki[4], A. Kraja[4], M. Province[4], J.S. Pankow[5], J.B. Wilk[6], J.E. Hixson[7], G. Heiss[1].* 1) Epidemiology, UNC, Chapel Hill, NC; 2) Genetics, UNC, Chapel Hill, NC; 3) Radiol Sci, Wake Forest Med, Winston-Salem, NC; 4) Biostat, Wash U Med, St. Louis, MO; 5) Epidemiology, U of Minn, Minneapolis, MN; 6) Neurology, BU Med, Boston, MA; 7) Hum Genetics, UTHSC, Houston, TX.

There is growing evidence that complex interactions among genetic and environmental factors influence the pathogenesis of atherosclerosis. In previous work in the NHLBI Family Heart Study (FHS), two QTLs for calcified aortic plaque, (CAP) on chromosomes 7p and 13q were localized. In this study, these analyses were extended to incorporate additive and QTL specific genotype-by-smoking interaction. Families in FHS were recruited from five US study centers. During standardized clinical exams smoking habits were ascertained and CAP was quantified with helical CT. MGS typed a total of 398 microsatellite markers. Among 2,133 Caucasians comprising 4,703 relationship pairs variance component linkage analysis in both in the full sample and samples stratified by smoking exposure dichotomized as ever/never, current/non-current, and, 15 or 20 pack-years/ 15 or 20 pack-years of smoking was performed in SOLAR. Marker allele frequencies derived from pedigree founders were used to estimate multipoint identity-by-descent sharing using GeneHunter. The mean value of CAP was 3246 in men and 1923 in women. Ever smoking was reported by 907 participants, whereas only 194 reported that they currently smoked. Adjusting rank transformed CAP for age, sex, and center, significant additive genotype-by-smoking interaction was detected for all measures of smoking exposure (all p 0.05). QTL specific genotype-by-smoking interaction was detected in smokers with 15 pack-years smoking exposure (maximum adjusted LOD = 4.3) on chromosome 4 at 102 cM and in smokers with 20 pack-years of smoking exposure on chromosome 2 at 264 cM (adjusted LOD = 3.1). These findings will be further tested in the African American FHS sample, upon completion of genotyping. Accounting for genotype-by-smoking interaction in the pathogenesis of CAP improves our ability to detect QTLs.

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Reliability of high throughput genotyping of whole genome amplified DNA in large-scale genotyping studies. *Y. Berthier-Schaad[1], W. Kao[1], L. Zhang[1], N. Fink[1], J. Coresh[1], M. Smith[2].* 1) Department of Epidemiology, Johns Hopkins University, Baltimore, MD; 2) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.

Whole genome amplification (WGA) of DNA has been proposed as the solution for limited DNA in human studies. Previous studies have examined the reliability of genotyping of WGA DNA samples, but they have focused on small number of SNPs or individual samples (N<5) or on samples derived from cell lines. We determined the reliability of WGA DNA genotypes in two sets of replicated samples: (1) WGA-original: 33 pairs of original DNA vs. WGA DNA and (2) WGA-WGA: 32 split samples of WGA DNA. WGA DNA samples were amplified using the multiple displacement amplification method (MDA). Genotyping was successful for 1362 of 1536 SNPs from 164 cardiovascular candidate genes in a single Illumina panel. Percent of successful SNP calls and kappa statistics () were calculated for all SNPs and by Illumina SNP assay score and gen_call score. In the 33 WGA-original pairs, mean call rate for original DNA samples was significantly higher than that of WGA samples (99% vs 93%). Approximately 5% of assays that were successfully typed in original samples were not in WGA samples. A total of 41,428 pairs of WGA-original genotype data were available for genotype concordance analysis. Mean for WGA-original pairs was 0.98. Concordance between WGA-original pairs was high irrespective of either SNP assay score or gen_call score. Reproducibility was lower amongst WGA-WGA pairs (mean for the 32 WGA-WGA pairs was 0.89; inter-quartile range: 0.84-1.00). However, the lower concordance was largely driven by 18 WGA-WGA pairs where the original DNA was not of sufficient quality or quantity for the Illumina platform. In summary, the MDA method produces WGA DNA samples that can be genotyped using high-throughput technology with slightly lower call rates but very high reproducibility to the original DNA. These results also indicate that variability in the original DNA quality plays a role in WGA reproducibility. This method of DNA amplification will be useful for future large-scale genetic analyses in studies with limited DNA.

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Can genes act as mediators describing the gene-environment interaction effects on obesity? *B.M. Chakraborty[1], M.B. Rao[2], R. Chakraborty[2].* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Center for Genome Information, Univ. Cincinnati, OH.

Gene-environment interaction (GxE) effect on phenotypes is described by heterogeneity of environmental (E) effects across different genotypes, or by genotypes (G) having different phenotypes under different environments. GxE effects may be significant even in the absence of main effects of G and/or E. Distinctions of different forms of GxE effects have been used in psychological and nursing studies by utilizing the concepts of mediators and moderators. Statistical methodologies (structural equation models and path diagrams) have been used to distinguish these two forms of interactions, but in the genetic context the explicit distinction of different forms of GxE effects has been limited. For example, in obesity research, twin/adoption studies suggest that 30-70% of the inter-individual variation of obesity may be due to underlying genetic variation, and more than 70 possible candidate gene loci have been implicated. These include genes involved in central nervous regulation systems (e.g., leptin, the leptinreceptor, POMC, MCR-4), and energy expenditure and adipocyte differentiation (e.g., beta-adrenergic receptors, UCPs, and the nuclear receptor PPARy). Many of these genes regulate food intake and energy expenditure. However, the three-way link between gene polymorphism, food intake/exercise habit, and obesity has not been rigorously studied to document the precise nature of GxE effects on obesity. Consequently, data on absence of obesity-causing mutations or sequence variation at the Ob-Rb, PYY, and Y2R genes, without considering the caloric ingestion and physical exercise characteristics of the subjects, cannot be properly interpreted. We provide a theoretical framework for testing the significant role of such gene polymorphisms using a model of mediating type of GxE interaction effects on obesity in which the main effect of genes may remain undetected without data on physical activity and/or caloric intake of subjects. Study designs for testing such interaction effects are illustrated with their relevant power computations. (Research supported by a NIH grant GM41399).

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Genomic screening and replication in one data set in family-based association testing. *C. Lange[1,3], K. van Steen[1], M. McQueen[1], A. Herbert[2], B. Raby[3], H. Lyon[5], D. DeMeo[3], A. Murphy[1], J. Su[1], S. Datta[3], C. Rosenow[4], M. Christman[2], E. Silverman[3], N. Laird[1], S. Weiss[3].* 1) Dept Biostatistics, Harvard Sch Public Health, Boston, MA; 2) Department of Genetics and Genomics, Boston University School of Medicine, Boston, Massachusetts 02115, USA; 3) Channing Laboratory, Harvard Medical School, Boston, Massachusetts 02115, USA; 4) Genomics Collaboration Genotyping, Affymetrix, Inc., Santa Clara, California 95051, USA; 5) Division of Genetics, Childrens Hospital, Boston, Massachusetts 02115.

The Human Genome Project and its spin-offs are making it increasingly feasible to determine the genetic basis of complex traits using genome-wide association studies. The statistical challenge of analyzing such studies stems from the severe multiplecomparison problem resulting from the analysis of thousands of SNPs. Our methodology for genome-wide family-based association studies, using single SNPs or haplotypes, can identify associations that achieve genome-wide significance. In relation to developing guidelines for our screening tools, we determined lower bounds for the estimated power to detect the gene underlying the disease-susceptibility locus, which hold regardless of the linkage disequilibrium structure present in the data. We also assessed the power of our approach in the presence of multiple disease-susceptibility loci. Our screening tools accommodate genomic control and use the concept of haplotype-tagging SNPs. Our methods use the entire sample and do not require separate screening and validation samples, as population-based designs do. We will illustrate the practical relevance of the proposed screening tools by an application to a genome-wide association study (100K-scan) of the Framingham Heart Study.

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Evaluation of analysis methods for whole genome association studies with 100K SNPs. *M.G. Ehm[1], S.-A. Bacanu[1], M.R. Barnes[2], C.Z. Bowman[1], L.P. Briley[1], J. Charnecki[1], O. Delrieu[4], R.A. Gibson[4], M.L. Jones[5], K. Long[3], M.R. Nelson[1], K.L. Nangle[3], M.V. Plumpton[2], S. Shouse[1], Z. Xue[1], D.P. Yarnall[1], E.H. Lai[1].* 1) Discovery & Pipeline Genetics, GlaxoSmithKline, Res Triangle Pk, NC; 2) Bioinformatics, GlaxoSmithKline, Harlow, UK; 3) Bioinformatics, GlaxoSmithKline, Res Triangle Pk, NC; 4) Translational Medicine & Genetics, GlaxoSmithKline, UK; 5) Bioinformatics Research Center, North Carolina State University, Raleigh, NC.

The commercial availability of Affymetrix Genechip technology (100K and 500K SNP arrays) has made it possible for whole genome association scans to be completed in research laboratories. To develop a capacity to efficiently complete the genotyping for whole genome scans, test the capacities of our bioinformatics systems, investigate analysis techniques, and interpret the results, we genotyped 126 unrelated Caucasian subjects including 63 patients and 63 controls using the 100K mapping set. We tested for association between this complex disease and SNPs on this chip using a variety of analysis methods, including Fishers exact allelic and genotypic tests. Interpretation of results was aided by clustering results using linkage disequilibrium. We also used visualization of gene and pathway determinants of disease using a novel application of empirically derived log Bayes Factors. We compared the results identified by these techniques and interpretation for whole genome association screens, in general. While this study was underpowered to identify modest effects, the information learned from this experiment will guide the further development of statistical methods for appropriately powered studies using the 500K mapping set.

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Single SNP vs haplotype block based association studies. *Z. Yu[1], R. Guerra[1], J.C. Cohen[2,3], H.H. Hobbs[2,3].* 1) Dept Statistics, Rice Univ, Houston, TX; 2) Donald W. Reynolds Cardiovascular Clinical Research Center and the Department of Internal Medicine, Dallas, TX; 3) Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX.

Recently, a lot of articles have discussed the power of single SNP and haplotype based association studies. Much of the discussion has been based on theoretical considerations or simulations. In this study we analyzed the association between several phenotypes and regions where SNPs were fully genotyped using both single-SNP and haplotype block based analyses. The data set is a stratified probability sample from Dallas County and represents three ethnic groups. The comparison between the two approaches was based on physical length, number SNPs and marker density of haplotype blocks. Advantages and disadvantages of each approach exist and described in this presenation.

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A quantitative genetic paradox of association studies involving quantitative trait and a genetic polymorphism.
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Association of genetic polymorphism with quantitative phenotypes is traditionally established by analysis of variance (ANOVA) of the quantitative trait with subjects classified by genotypes. Trait values are sometimes transformed to conform to the assumption of normality when the sampled subjects are biologically unrelated. With the other implicit assumptions satisfied (e.g., there is no deviation of Hardy-Weinberg expectation of genotype frequencies, and covariate of phenotypes are adjusted for), the most likely interpretation of genotypic association with phenotype variation is that the locus studied is at linkage disequilibrium (LD) with a putative major gene explaining a significant proportion of the phenotypic variation in the sampled subjects. Using the quantitative genetic model of major genes, we show that this method of analysis poses a quantitative genetic paradox. Analytical derivations suggest that the critical assumption of ANOVA, namely, homogeneity of the variance of phenotypic values across genotypes, is inherently violated in this model, unless the locus itself is the causative locus. The differences of within genotype variance of the trait are composite functions of LD, allele frequencies, and allelic affects on the phenotype. In fact, the homoscedasticity assumption of ANOVA is satisfied only when the LD is complete, or the null hypothesis of no major gene effect is true. In other words, the paradox arises from the fact that the critical assumption of ANOVA is always violated when the null hypothesis is not true, but the causative locus is not the one examined. With parameter estimates from some phenotype-gene association studies (e.g., APOE gene effect on lipid variation), we provide a numerical strength of the violation of the assumption. We propose a mixture analysis methodology to handle such quantitative genetic paradox of association studies of quantitative traits with gene polymorphism. (Research supported by a NIH grant GM41399).

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The A1298C and C677T polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene in Mexican patients with rheumatoid artritis (RA). *J. Mena[1], I. Davalos[1], J.I. Gamez[2], L. Gonzalez[2,3], F. Muñoz-Valle[4], M. Vasquez del M[4], G. Martinez[4], L. Sandoval[1], H. Rangel[5], M. Casillas[1], L.E. Figuera[1], M. Salazar-Paramo[2,4].* 1) Division de Genetica, CIBO, IMSS, y Doctorado en Genetica, U de G; 2) UIMEC, UMAE, HE, CMNO, IMSS; 3) HGZ 110, IMSS; 4) Departamento de Fisiologia,U de G; 5) CUCienega, Universidad de Guadalajara, Jalisco, Mexico.

Introduction. The enzyme methylenetetrahydrofolate reductase plays a critical role in homocysteine metabolism, catalyzing the conversion of 5,10- methylenetetrahidrofolate to 5-methylenetetrahydrofolate, the methil group donor in the B12 - dependent remethylation of homocysteine to methionine. Two common variants in the MTHFR gene (C677T and A1298C) are associated with reduced MTHFR specific activity. Moderate hyperhomocysteinaemia and folate deficiency has been described in patients with autoimmune diseases including RA. Objective. To determine the genotype frequency (GF) and allelic frequency (AF) of MTHFR C677T and A1298C polymorphisms in Mexican patients with RA. Methods. Group RA consisted in 119 RA patients diagnosed according to the American College of Rheumatology (ACR) and group M consisted in 140 normal individuals. Both groups were typed for the MTHFR C677T and A1298C by PCR/RFLP method. Results. MTHFR C677T GF (n) in the RA group were: CC 26 (31), CT 59 (70), TT 15 (18) ; AF (n): C 56 (132) and T 44 (106). The GF In the group M were: CC 31 (44), CT 52 (72), TT 17 (24); AF: C 57(160) and T 43(120). MTHFR A1298C GF in the RA (n=79) group were, AA 61 (48), AC 34 (27), CC 5 (4); and the AF: A 78 (123) C 22 (35). For group M (n=43) AA 49 (21), AC 46(20), CC 5(2); AF: A 72(62) C 28(24). Genotype frequencies were in agreement with the HWE. Conclusion. There were similar MTHFR C677T and A1298C AF between the two groups.

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Quantitative Analysis of the CYP2A6 gene deletion using pyrosequencing. *D. Koontz, A. Spencer, S. Nikolova, J. Huckins, M. Gallagher.* CDC, DLS, NCEH, Molecular Biology Branch, Atlanta, GA.

CYP2A6 plays a major role in the metabolism of drugs and precarcinogens, in particular nicotine and coumarin. Numerous polymorphisms in this gene have been characterized and contribute to much of the interindividual variability in CYP2A6 activity. One such variant is a total deletion of the gene (CYP2A6*4) and is found at a high frequency in the Japanese population. This gene deletion has been associated with lowered risk for tobacco-related cancers due to impaired ability to activate tobacco smoke precarcinogens. To date, the method for genotyping CYP2A6*4 has been by PCR-RFLP which involves enzyme digestion of a CYP2A6-specific gene fragment to generate banding patterns specific for the presence or absence of the gene. This method is laborious and not easily adapted for studying large numbers of samples. The proposed mechanism for this gene deletion involves an unequal crossover event between homologous regions of CYP2A6 and the CYP2A7 pseudogene. We exploited resulting sequence differences between the downstream region of CYP2A6 and CYP2A7 in order to design a pyrosequencing assay that would easily genotype homozygous normal, heterozygous, and homozygous gene deletion. Genotype calls were derived from informative raw peak height ratios that give unique values for each genotype. We genotyped a Japanese panel (N=10) and a multi-ethnic panel of 90 individuals (N=24 European American, N=24 African American, N=12 Mexican American, N=6 Native American, N=24 Asian American) both from the Coriell Human Variation Collection; results showed a CYP2A6*4 allele frequency of 15% [one homozygote and one heterozygote(3/20)] and 4% [eight heterozygotes (8/180)], respectively. This striking inter-ethnic difference in the distribution of the CYP2A*4 allele is consistent with previous reports of a much higher prevalence in Japanese populations. All results were 100% concordant with conventional PCR-RFLP. Here we present a rapid and reliable assay to assess CYP2A6*4 that lends itself to large-scale studies needed to accurately assess the prevalence of this gene deletion in populations and to generate informative genotype-phenotype correlations.

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Genomic Variation in Transforming Growth Factor Beta 1 Induced Transcript 1 (TGFB1I1) is Associated with Multiple Measures of Body Composition in Afro-Caribbean Men. *A.L. Kuipers[1], S.P. Moffett[1], C.S. Nestlerode[1], V.W. Wheeler[2], A.L. Patrick[2], C.H. Bunker[1], J.A. Cauley[1], R.E. Ferrell[1], J.M. Zmuda[1].* 1) Epidemiology, University of Pittsburgh, Pittsburgh, PA; 2) The Tobago Health Studies Office, Scarborough, Tobago, West Indies.

Transforming growth factor beta 1 induced transcript 1 (TGFB1I1) is a coactivator of nuclear receptors such as the androgen receptor and PPARgamma. We performed genetic association analyses of TGFB1I1 and body composition related phenotypes by genotyping a C-to-G substitution in the 3 untranslated region of TGFB1I1 (rs10596) in 925 Afro-Caribbean men aged 40 years and older. Body composition was measured with dual-energy X-ray absoprtiometry (Hologic QDR 4500). The frequency of the minor G allele was 0.101. Genotype frequencies were: CC, 89.84 %; CG, 9.95 %; and GG, 0.22% and did not depart from Hardy Weinberg Equilibrium. Presence of the G allele was associated with increased body weight (CC: 82.20 0.51 kg; CG+GG: 86.84 1.52 kg; p = 0.004) and height (CC: 174.1 0.2 cm; CG+GG: 175.6 0.6 cm; p = 0.019). The increased body weight associated with the G allele was due to increased lean mass (CC: 63.08 0.28 kg; CG+GG: 65.55 0.84 kg; p = 0.006) and fat mass (CC: 16.85 0.24 kg; CG+GG: 18.74 0.72 kg; p = 0.013). Carriers of the G allele also had significantly increased femoral neck bone mass (CC: 0.971 0.005 g/cm2; CG+GG: 1.003 0.015 g/cm2; p = 0.0384). The association with bone mass was no longer statistically significant after adjusting for the increased body weight and height among carriers of the G allele. Our preliminary analyses suggest that genomic variation at the TGFB1I1 locus may contribute to phenotypic variation in body size and body composition related traits. Studies with additional markers spanning the locus and in other samples are required to confirm and extend this hypothesis.

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Nuclear Receptor Coactivator-3 Alleles are Associated with increased bone mass in Afro-Caribbean men. *Y. Sheu[1], J. Zmuda[1], S. Moffett[1], C. Bunker[1], J. Cauley[1], A. Patrick[2], C. Ishwad[3], R. Ferrell[3].* 1) Epidemiology, University of Pittsburgh, Pittsburgh, PA; 2) Tobago Health Studies Office, Scarborough, Tobago, West Indies; 3) Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Sex steroid hormones are an important determinant of bone health in men. Nuclear receptor coactivator-3(NCOA3) is a steroid hormone receptor coactivator that facilitates steroid hormone receptor transcriptional activation of target genes. NCOA3 may affect bone mineral density(BMD) by modulating inter-individual differences in skeletal sensitivity to sex steroid hormones. To test this hypothesis, we conducted genetic association analyses between potentially functional alleles at this locus, body composition, and whole body and hip BMD(Hologic QDR450) in 814 Afro-Caribbean men(age 6110 yrs, mean SD; range 40-84 yr). Men were genotyped for a CAG repeat polymorphism in NCOA3, which encodes a polyglutamine tract of variable length in the C-terminal transcriptional activation domain of the protein. We found a significant increase in both hip and whole body BMD with increasing copies of the most common allele(224bp, 43.2%) before and after adjusting for age, weight and height. For example, men without the 224bp allele had 5% lower femoral neck BMD than men who were homozygous for this allele(Table). Similar results were observed for whole body BMD. No significant differences were found for whole body fat mass and lean mass across genotypes. We conclude that allelic variation at the NCOA3 locus may contribute to the genetic control of bone mass among older men.

Table:BMD(g/cm2) by number of copy of NCOA3 Allele.

	0(n=204)	1(n=516)	2(n=94)	p-value
Whole Body	1.23(.12)	1.27(.11)	1.28(.12)	.0005
Femoral Neck	.94(.15)	98(.14)	.99(.15)	.0013

Values are means (SD) and adjusted for age, weight and height.

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The ATGL gene is associated with free fatty acids, glucose and trigylceride concentrations. *F. Kronenberg[1], V. Schönborn[1], I.M. Heid[2], C. Vollmert[2], A. Lingenhel[1], T.D. Adams[3], P.N. Hopkins[3], T. Illig[2], R. Zimmermann[4], R. Zechner[4], S.C. Hunt[3].* 1) Div. of Genetic Epidemioloy, Innsbruck Medical University, Innsbruck, Austria; 2) GSF-National Research Center for Environment and Health, Insitute of Epidemiology, Neuherberg, Germany; 3) Cardiovascular Genetics, University of Utah, Salt Lake City, UT; 4) Institute of Molecular Biosciences, Karl-Franzens-University, Graz, Austria.

Adipose triglyceride lipase (ATGL) was recently described to predominantly perform the initial step in triglyceride hydrolysis (Science 306:1383, 2004) and therefore seems to play a pivotal role in the lipolytic catabolism of stored fat in adipose tissue. This is the first study which investigates genetic variations within the ATGL gene in humans. We investigated in a large epidemiological study involving 2434 Caucasians from Utah, USA, twelve polymorphisms in the ATGL gene identified via sequencing and data base search. These polymorphisms and their statistically reconstructed haplotypes were analysed for association with plasma free fatty acids (FFA) and other phenotypes related to lipoprotein metabolism, obesity, and type 2 diabetes. FFA concentrations were significantly associated with several SNPs and haplotypes of the ATGL gene (decreased FFA levels: SNPs 3,6,8,10,11,12, p-values from 0.016 to 0.00007), consistent with additive inheritance. Three rare SNPs (SNPs 5,7,9) showed a tendency towards significance. Furthermore, SNP5 (and similarly SNP9) showed associations with glucose levels (p=0.00017, +19 mg/mL), risk of type 2 diabetes (OR=2.6, p=0.025) and nonsignificant but consistent differences in BMI and HOMA. Triglyceride levels showed a nonsignificant tendency for an association with ATGL in accordance with the FFA associations. The associations of triglycerides and glucose became stronger when restricting the analysis to subjects without diabetic medication. Haplotype analysis supported and extended the shown SNP association analyses. In summary, genetic variation within the ATGL gene shows strong associations with FFA, and less pronounced but still detectable associations with parameters of the metabolic syndrome and type 2 diabetes.

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A regression-based association approach using haplotype similarity for genome scans. *J.Y. Tzeng[1], M. Davidian[1], D.C. Thomas[2].* 1) Department of Statistics, North Carolina State Univ, Raleigh, NC; 2) Division of Biostatistics, Department of Preventive Medicine, University of Southern California, Los Angeles, CA.

The prospect of genome-wide association scans is being enhanced by the progress in human genome research and the advance in the large-scale genotyping technique. In genome screening, chromosomal regions are divided into hundreds or thousands of small segments by forming a moving window, and association test are performed within each window to detect susceptibility locations. Commonly used approaches to test for association include using likelihood ratio tests or chi-squared tests of homogeneity, or regressing trait values on haplotypes. As the power of a single haplotype-based association test can be limited by its large degrees of freedom, the overall performance of an association scan can be further diminished by the multiple testing adjustment. To tackle this problem, we propose a regression-based approach for testing association using haplotype similarity; specifically we extend the haplotype test based on pair-wise similarity measures to a model based framework. We detect association by looking for the unusual sharing of chromosomal segments within homogeneous trait groups using a much fewer parameters, and extra degrees of freedom are spared by not modeling individual haplotypes. We use regression framework to accommodate environmental covariates, polygenic effects and corresponding interactions, and also gain flexibility in modelling different trait types. The method is connected with the ordinary regression that treats individual haplotypes as covariates under a hierarchical modeling framework. Through simulation studies we assess the performance of the proposed method, and demonstrate its validity in testing for genetic association.

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Association of SNP haplotypes of interleukin-1 beta with persistent sciatica following lumbar diskectomy. *T. Wu[1], A. Kingman[1], A. Bollettino[1,2], H. Hipp[1,2], M. Scully[1], I. Belfer[1,2], S. Atlas[3], B. Buzas[1,2], C. McKnight[1,2], R. Keller[4], D. Goldman[2], M. Max[1].* 1) National Institute of Dental and Craniofacial Research, Bethesda, MD; 2) National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD; 3) Massachusetts General Hospital, Harvard Medical School, Boston, MA; 4) Maine Spine and Rehabilitation Center, Scarborough, ME.

Interleukin-1 (IL-1) is a cytokine that is produced in response to infection, injury, or antigenic challenge. It mediates neuropathic pain behaviors in animal models. Solovieva et al.(2004) reported that IL-1 C3954T and disc degeneration and low back pain were likely associated. We examined association of 10 single nucleotide polymorphisms (SNPs) in IL-1 gene with leg pain (z-score) in 107 Caucasian patients, who had been followed for 10 years after lumbar diskectomy for sciatica in Maine Lumber Spine Study (Atlas et al., 2001) . A Z-score was calculated for each patient based on the four leg pain scores (7-point questionnaires about frequency and post-surgical improvement in leg pain and leg pain after walking) at baseline, 3, 6, 12 and 24 months. Strong linkage disequilibrium among the 10 SNPs was observed, the pair-wise D ranging from 0.82 to 1.00. Five haplotypes were inferred from the population by EM algorithm (SAS/Genetics). Analysis of individual SNPs indicated that none of the ten SNPs showed significant association with the leg pain z-score. Haplotype-based analysis revealed that the haplotype CGCGAAGCGT(with frequency 13.7%) had the highest adjusted mean of z-score among the five haplotypes. A multilocus diplotype-based analysis found patients with the haplotype had significantly higher pain z-scores than those without the haplotype (p=0.018). The result suggests that polymorphisms in the IL-1 gene may affect the risk of chronic sciatica after lumbar diskectomy. 1 Solovieva, et al., Pain, 2004. 2 Atlas, et al., Spine, 2001.

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Estimation and testing of genotype and haplotype effects in case/control studies: comparison of weighted regression and multiple imputation procedures. *H.J. Cordell.* Dept of Medical Genetics, University of Cambridge, UK.

A popular approach for testing and estimating genotype and haplotype effects associated with a disease outcome is to conduct a population-based case/control study, in which haplotypes are not directly observed but may be inferred probabilistically from unphased genotype data. A variety of methods exist to analyse the resulting data while accounting for the uncertainty in haplotype assignment, but most focus on the issue of testing the global null hypothesis that no genotype or haplotype effects exist. A more interesting question, once a region of disease association has been identified, is to estimate the relevant genotypic or haplotypic effects and to perform tests of complex null hypotheses such as the hypothesis that some loci, but not others, are associated with disease. Here I examine the performance of two classes of methods for addressing this question. The first is a weighted regression approach in which posterior probabilities of haplotype assignments are used as weights in a logistic regression analysis, generating a test based on either a weighted pseudo-likelihood, or a weighted log-likelihood. The second is a multiple imputation approach using either an improper procedure in which the posterior probabilities are used to generate replicate imputed data sets, or a proper data augmentation procedure. In simulations, all methods were unbiased but the weighted pseudo-likelihood and multiple imputation methods had superior confidence interval coverage. Multiple imputation was easier to implement in standard statistical software and to extend to more complex models of gene-gene or gene-environment interaction.

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Normalized logistic-based rank transformation for QTL mapping methods. *K. Shibata[1], R. Takemura[2], N. Kamatani[2,3].* 1) Integrated Database Group, Japan Biological Informatics Consortium, Tokyo, Japan; 2) Algorithm Team, Japan Biological Informatics Consortium, Tokyo, Japan; 3) Tokyo Women's Medical University, Tokyo, Japan.

QTL mapping is generally based on the assumption that the quantitative phenotypes follow normal distributions. However, many real phenotypes are not normally distributed. One approach is to attempt to find a mathematical transformation that will convert the trait into a normal distribution for genetic mapping of quantitative trait loci. However, given a particular transformation such as Box-Cox transformation, we need to estimate the optimal value of the transformation parameter. Also, the effect of outliers may be too great and an appropriate transformation may be difficult to find. Here, we introduce the logistic-based rank transformation for QTL mapping, which does not involve the optimal choice of parameters. We define the ranks of the observed quantitative phenotypes, where the ranks are 1 for the smallest observation, 2 for the next, preserving the order of the data. Our strategy is to transform the order of the data into an approximate normal distirbution using logistic function, which is implemented in the latest version of QTLHAPLO (Shibata et al. 2004). We conduct the simulations based on real data and the phenotypic effect for multivariate phenotypes can be estimated. We discuss the effect of logistic-based rank transformation on power and type I error rates of parametric association test where the phenotype has a Cauchy distribution, uniform distribution and exponential. We have compared the power of the association study under different modes of inheritance. The logistic-based rank transformation represents an important initial step in QTL analysis under a variety of distributions.

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An Algorithm for Constructing Associated Haplotypes from High Density SNP Genotype Data. *J.M. Laramie[1], J.B. Wilks[2], A.L. DeStefano[3], R.H. Myers[2].* 1) Bioinformatics, Boston University, Boston, MA; 2) Departments of Neurology, Boston University School of Medicine, Boston, MA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA.

Introduction We present an algorithm called *HaploBuild* that, in conjunction with the program haplotype FBAT (Family Based Association Tests)[1], can construct nonconsecutive haplotypes of any length that are statistically associated, through a family based association test, with a quantitative or qualitative phenotype from a set of high density single nucleotide polymorphism (SNP) and microsatellite genetic markers.

Methods In essence, the *HaploBuild* algorithm defines a function for choosing markers that are combined as a haplotype and tested for association with a disease phenotype. Given a set of genotyped markers our algorithm works in two steps. The first step tests for association all two marker haplotypes within a given distance of each other. The second step builds an *n-ary* tree from each of the two marker haplotypes that reached a specified level of significance in Step 1. The goal of tree building is to greedily add markers one at a time to a haplotype increasing the overall haplotype association significance and pruning those nodes that do not increase significance. In this context, the leaf nodes of the trees built represent the maximal number of markers that can be combined into an significantly associated haplotype.

Discussion In a simulation study, *HaploBuild* correctly finds the haplotypes containing the SNP causing the phenotypic effect. If the SNP causing the phenotypic effect is removed prior to analaysis *HaploBuild* finds haplotypes in strong LD with the removed SNP. In our simulation study we were able to narrow the canidate gene region from 18Mb down to ~400kb.

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Haplotype analysis in the presence of missing data. *N. Liu[1], I. Beerman[2], R. Lifton[2], H. Zhao[1,2].* 1) Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; 2) Department of Genetics, Yale University School of Medicine, New Haven, CT.

It is common to have missing genotypes in practical genetic studies, and the exact underlying missing data mechanism is generally unknown to the investigators. Although some statistical methods can handle missing data, they all assume that genotypes are missing at random, that is, at a given marker, different genotypes and different alleles are missing with the same probability. These include those focusing on haplotype frequency estimation and haplotype association analysis. However, it is likely that this simple assumption does not hold in practice, yet no study to date has examined the magnitude of the effects when this simplifying assumption is violated. In this study, we demonstrate that the violation of this assumption can lead to serious bias in haplotype frequency estimates, and haplotype association analysis based on this assumption can induce both false-positive and false-negative evidence of association. To address this limitation in the current methods, we propose a general missing data model to characterize missing data patterns across a set of two or more markers simultaneously. We prove that haplotype frequencies and missing data probabilities are identifiable if and only if there is linkage disequilibrium between these markers under our general missing data model. Simulation studies on the analysis of haplotypes consisting of two single nucleotide polymorphisms illustrate that our proposed model can reduce the bias both for haplotype frequency estimates and association analysis due to incorrect assumption on the missing data mechanism. Finally, we illustrate the utilities of our method through its application to a real data set.

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New correction method for association study using SNPs in linkage disequilibrium with each other. *A. Takahashi[1], S. Fujii[2], J. Takasaki[1], T. Nakamura[1], M. Yanagisawa[2], Y. Nakamura[1], N. Kamatani[1,3].* 1) SNP Research Center, RIKEN, Yokohama, Kanagawa, Japan; 2) Department of Computer Science, Waseda University, Japan; 3) Institute of Rheumatology, Tokyo Woman's Medical University, Japan.

Single nucleotide polymorphisms(SNPs) have been effective markers to discover genes associated with diseases. If association studies using SNPs are performed over the whole genome, the number of association tests will be too large. Then the problem of multiple tests will emerge. Several methods have been proposed till now to overcome this problem including the standard Bonferroni's correction. It is well known, however, that Bonferroni's correction gives too conservative conclusions especially when SNPs are in linkage disequilibrium with each other. We propose a new correction method for case-control association studies using SNPs in linkage disequilibrium with each other. The equation of probability of exact type I error was derived given population haplotype frequencies. Exact type I error rate could be calculated numerically when sample sizes were small; however, it was difficult when the sample sizes were large. Here, we developed a Markov chain Monte Carlo method to calculate asymptotic type I error rate for large samples sizes.

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fastPHASE: an efficient method for haplotype reconstruction and missing genotype imputation using a flexible cluster-based model for population genetic data. *P. Scheet, M. Stephens.* Statistics, University of Washington, Seattle, WA.

Various methods exist for the analysis of haplotype data from unrelated individuals; however, studies of human genetic variation generally consist of unphased genotypes, where the haplotypes are not observed. With the advent of technologies for cheaply and quickly producing large amounts of genetic data, there is a coming need for models that are computationally tractable for genotype data from thousands of individuals at thousands of loci. Here we present such a model for genetic variation based on clustering the latent haplotypes over short regions. Existing approaches divide the data into discrete windows, but we take a more flexible approach that allows the clustering to change continuously along the chromosome according to a hidden Markov model for cluster membership. Parameter estimation is accomplished via an expectation maximization algorithm, and quantities of interest are estimated by averaging over multiple modes of the likelihood surface. We apply the model to dense SNP genotype data from real populations for the tasks of imputing missing genotypes and inferring haplotypes, and compare it against several existing methods. For imputing missing genotype data, performance is superior to the best available methods. And for haplotype reconstruction, our model performs almost as well as the best existing method but at a fraction of the computational cost. Our new methods for these tasks have been implemented in a software program called fastPHASE.

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Type I error and power for statistical association tests in the presence of haplotype misclassification. *M. Levenstien, J. Ott, D. Gordon.* Laboratory of Statistical Genetics, Rockefeller University, New York, NY.

Haplotype association studies are gaining popularity as a method for fine mapping susceptibility genes for human disease. Because current molecular haplotyping methods are expensive and not amenable to automation, many researchers rely on statistical methods to infer haplotype pairs from multilocus genotypes and subsequently treat these inferred haplotype pairs as observations. Although these methods provide an efficient alternative to molecular haplotyping, some haplotype pairs can be misclassified. We examine the effect of these misclassification errors on the type I error and power for a variety of association tests. These tests include the standard likelihood ratio test (standard LRT), a likelihood ratio test which weights the inferred haplotype pairs by the posterior probability associated with them (weighted LRT), and a likelihood ratio test that employs a double sampling approach to allow for the misclassification inherent in the haplotype inference procedure (LRTae). In addition, we aim to determine the cost-benefit relationship of increasing the double sample proportion (the proportion of individuals with molecular haplotype measurements in addition to genotypes) to raise the power gain of the LRTae over the standard LRT. This analysis should provide a guideline for determining the minimum number of molecular haplotypes required for desired power. Our simulations with two SNP markers indicate that 1) for each statistic permutation methods maintain the correct type I error; 2) complete misclassification occurs in the vast majority of situations; and 3) asymptotic p-values for the standard LRT appear to fluctuate between conservative and anti-conservative values.

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Dissecting the relationship beetween C-reactive protein and metabolic syndrome. Common *CRP* haplotypes: application of Medelian ranomisation. *N. Timpson[1], D. Lawlor[1], R. Harbord[1], T. Gaunt[3], I. Day[1, 3], L. Palmer[1, 2], et al[1, 4, 5].* 1) Dept of Social Medicine, Bristol University; 2) Western Australia Inst of Medical Research, UWA; 3) Human Genetics Research Division, University of Southampton; 4) Peninsular Medical School, Exeter University; 5) Medical Department, Haemostasis and Thrombosis Unit, Glasgow University.

CRP is associated with components of metabolic syndrome, but the nature of this relationship remains unclear. With common *CRP* haplotypes associated with plasma CRP, Mendelian randomisation was used to generate estimates of association between plasma CRP and metabolic syndrome phenotypes(MSPs) free from confounding/reverse causation. CRP associated SNPs were genotyped in 3218 British women and associations of *CRP* haplotypes with MSPs were assessed. Comparison was made between estimates of association between plasma CRP and MSPs derived from basic regression analysis and those from a Mendelian randomisation framework implemented via instrumental variables(IV) techniques. There were strong linear associations between log plasma CRP concentrations and MSPs (all p*0.001). Differences existed between mean circulating CRP for observed haplotypes (p=*0.001). Haplotypes CAC and GGT (rs1800947/rs1130864/rs1205) showed strongest association with higher and lower plasma CRP levels respectively (geometric means for plasma CRP: CAC 2.03[2.02, 2.04]; GGT 1.39[1.38, 1.40]). Potential confounders, e.g. smoking, were associated with plasma CRP concentration (all p=*0.003), but don't show evidence for association with *CRP* haplotypes. There was strong evidence that the two methods of estimating the CRP/MSPassociations gave conflicting results for the CRP-BMI association (p=0.0002) and evidence of conflicting results for associations of CRP with HOMA-R (p=0.014), Triglycerides (p=0.03) and HDLc (p=0.07). We observed disparity in estimates of the relationship between plasma CRP and MSP derived from conventional analyses and an IV approach, This suggests no association between the component of CRP directly related to *CRP* variation and MSPs. Analyses are analogous to an RCT of plasma CRP levels and do not suggest a causal role for CRP in the aetiology of MSPs.

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Haplotype reconstruction in population individuals using coalescent trees. *D. Qian[1], L. Beckmann[2].* 1) Department of Biostatistics, City of Hope National Medical Center, Duarte, CA, USA; 2) Division of Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany.

We present a two-stage algorithm to reconstruct haplotypes in population individuals using hierarchical clustering and coalescent tree analysis. First, haplotypes are grouped into clusters, and each cluster contains similar haplotypes that are likely descended from a common ancestor. Second, haplotypes within each cluster are fitted to a coalescent tree structure. Each tree is then quantified with a tree distance, and the haplotype configuration with the minimum sum of tree distances is defined as the optimal one under the identified trees. The similarity between haplotypes as well as the tree distances are calculated based on haplotype sharing, i.e. the number of shared markers identical by state. The approach allows for multiple origins of haplotypes within a population. The plausibility of a haplotype is quantified using similar haplotypes within the same cluster, and the effect of random similarities between clusters has been excluded. The algorithm is applicable for both SNPs and multiallelic microsatellites.

We analyzed three real data sets with known molecular haplotypes. The impact of missing data was analyzed in a simulation study by imputing randomly 3% and 6% missings into the data sets. For comparison, we used three commonly used algorithms (PHASE, HAPLOTYPER, SNPHAP). Preliminary results indicate similar performance as the Bayesian approach implemented in PHASE. Our approach outperformed HAPLOTYPER and SNPHAP in the presence of missing data in two data sets.

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Associations of the apolipoprotein A1/C3/A4/A5 gene cluster with triglyceride and HDL levels in women with type 2 diabetes. *L. Qi[1,2,4], S. Liu[1,2,4], N. Rifai[3], D. Hunter[1,2,4], F. Hu[1,2,4].* 1) Department of Nutrition, Harvard School of Public Healt, Boston, MA; 2) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 3) Department of Laboratory Medicine, Childrens Hospital and Harvard Medical School, Boston, MA; 4) Channing Laboratory, Department of Medicine, Brigham and Womens Hospital and Harvard Medical School, Boston, MA.

Recent studies have shown that the apolipoprotein gene cluster (APOA1/C3/A4/A5) contributes to the homeostasis of triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) and may affect the risk of coronary heart disease (CHD) in non-diabetic populations. Patients with type 2 diabetes are prone to accelerated atherogenesis. However, no study has investigated the association of the genetic variability in APOA1/C3/A4/A5 cluster and the levels of TG and HDL-C among diabetic patients. We examined the effects of ten polymorphisms at APOA1/C3/A4/A5 gene cluster in 902 women with type 2 diabetes. A linkage disequilibrium (LD) breakdown was observed between APOA5 and other genes. Two common haplotypes encompassing four APOA5 polymorphisms (SNP1, SNP2, S19W, and SNP3) were significantly associated with 37% (haplotype 2212, APOA5*2, P=0.016) and 60% (haplotype 1121, APOA5*3, P=0.0002) higher fasting TG levels as compared with the most common haplotype (1111, APOA5*1) respectively. Adjustment for age, BMI, and other covariates did not appreciably change such associations. The homozygosity of APOC3 promoter polymorphism, -455T/C, was also associated with significantly increased fasting TG levels (P=0.012). The effects of APOA5 and APOC3 were independent of each other. In addition, APOA4 347T/A was associated with significantly lower levels of HDL-C under a dominant inheritance model (P=0.009). Our results indicate that the variability of APOA5 and APOC3 gene may independently affect fasting TG levels, and, variants at APOA4 locus may affect HDL-C levels in women with type 2 diabetes.

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Disease gene location estimation using admixture population. *S. Zhang[1], Q. Sha[1], X. Zhu[2].* 1) Dept Mathematical Sci, Michigan Tech Univ, Houghton, MI; 2) Department of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL.

Admixture mapping is an efficient approach to localizing disease-causing variants that differ in frequency between two historically separated populations. It may be more powerful than linkage studies and, for a genome search, it typically require only ~1%; genotyping effort as many as required by a genome-wide association study. Recently, several methods have been proposed to test linkage between a susceptibility gene and a disease locus by using admixture-generated linkage disequilibrium (LD) for each of the typed markers. In this report, we propose a likelihood based method to estimate the location of disease genes which include point estimation and interval estimation. The method is applicable to a candidate region or a genome-wide search under either a case-control design or a case-only design. We use simulation studies to evaluate the performance of the method. In simulation studies, we generate admixture population under different population models by using a dense ancestry-informative marker penal for African-American. The simulation results show that the proposed method approximately gives an unbiased location estimation and the coverage of 95%; confidence interval is approximately 95%.

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Nonparametric Bayesian models for family-based association tests with multi-allelic markers and gene-gene interactions. *B. Shahbaba[1], S. Malik[2], E. Schurr[2], C.M.T. Greenwood[3].* 1) Public Health Sciences, University of Toronto, Toronto, Canada; 2) Research Institute of the McGill University Health Centre, Montreal, Canada; 3) Genetics & Genomic Biology, Hospital for Sick Children, Department of Public Health Sciences, University of Toronto, Toronto, Canada.

Genetic association studies, particularly for the family-based control design, are plagued by small sample sizes and sparse data. In particular, when examining gene-gene interactions, the number of informative parents may be extremely small. Here we propose a nonparametric Bayesian model for analyzing genetic associations in trios of an affected child and two parents. We use continuous latent variables for modeling multivariate categorical data. By transforming the categorical variables into a continuous space, we overcome some problems associated with estimating numerous parameters in a sparse contingency table. Our approach is based on finding a set of latent variables which, when conditioned upon, the categorical variables (genotypes or haplotypes) can be regarded as independent. In this way, any dependency between the observed categorical variables can be induced based on dependency between the latent variables. We model the joint distribution of the latent variables as a mixture of simple multivariate normal distributions. The mixing distribution is given a Dirichlet process prior. Markov chain Monte Carlo methods are used for estimation. Missing information is a ubiquitous problem in statistics. Here, we model the joint distribution of response and all predictors. As a result, we avoid direct imputation of missing values by assuming they occur at random. This approach is illustrated using data collected on three candidate genes among 110 families ascertained to have a child with tuberculosis. Genetic markers are measured in parents and their affected child(ren). Our results show that one of the genes (Vitamin D Receptor) may have a small effect on risk; no interactions were identified. Using standard methods, however, testing for interactions is difficult.

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A unified multilocus association test. *H.C. Yang[1], C.Y. Lin[2], C.S.J. Fann[1,2].* 1) Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Public Health, Yang-Ming University, Taipei, Taiwan.

Multilocus association tests using information of inter-marker linkage disequilibrium is more powerful than single locus tests when disease etiology is contributed by a simultaneous work of several linked genetic loci related to each other. However, reduction of testing power or inflation of type 1 error may be caused by noisy interference due to the use of non-informative markers, demonstrating the importance of marker selection for multilocus association mapping. In this study, we proposed a unified association test based on a convenient marker-selection procedure (sliding window) combined with an adjustment approach (SNP weighting) to diminish the impact of non-informative markers on association tests. The new procedure includes several conventional p-value combination methods as its special cases, where sampling distributions of the test statistics were discussed. Comprehensive simulation studies based on different evolutionary and disease models were performed to investigate the impact of nuisance markers and evaluate the performance of the suggested procedure. Simulation results show that testing power was inversely proportional to the quantity of nuisance markers. Among a class of p-value combination methods, the product p-value method had the highest testing power. Compared with conventional unweighted approach, the proposed weighted strategy not only improved testing power, but also reduced type 1 error. Two authentic data sets of disease gene mapping studies, psoriasis and Alzheimer disease, were analyzed. The results using the proposed method were consistent with the previous findings; more importantly, the proposed method is able to identify novel disease genes.

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A comparison of methods for evaluating the relative effects of neighbouring polymorphisms associated with a disease. *J.M. Biernacka, H.J. Cordell.* Dept. of Medical Genetics, Univ. of Cambridge, Cambridge, United Kingdom.

In a small region several marker loci may be associated with a trait. Some of these may be associated because they directly influence the trait, while others are associated because they are in linkage disequilibrium (LD) with a causal variant. Several methods have been proposed to assist in distinguishing potentially causal polymorphisms from those that show association due to LD. Given a postulated effect at a primary variant, we may ask if any other variants in the region appear to further contribute to the trait. Evidence of such further effects indicates that the additional variant is causal or is in LD with another causal locus. Subsequent conditioning on several markers may identify additional causal polymorphisms. Various methods of approaching this problem using case-parent trio data have been proposed, among those the stepwise conditional logistic method described by Cordell and Clayton (2002), and a permutation-based method recently proposed by Spijker et al. (2005). The test statistic proposed by Spijker et al. (2005) is a marginal test of association that does not in itself take into account previously identified polymorphisms. However, significance of this statistic is evaluated using a permutation procedure that does take into account previously identified loci. We compared these methods and other related approaches by simulation. Under the conditions investigated, no advantage was found using the permutation procedure over asymptotic conditional logistic regression. The procedure described by Spijker et al. (2005) and unconditional logistic regression can lead to inflated type 1 errors in situations when haplotypes are not inferable for all trios. Because these procedures rely on AFBAC (affected family-based) controls, they are prone to bias and therefore increased type 1 error when haplotypes cannot be inferred for all families, as illustrated in our simulations. We propose an alternative to the permutation method of Spijker et al. (2005), which does not rely on haplotyping, and thus avoids the bias inherent in procedures that use AFBAC controls. Our results show that this alternative procedure leads to good type 1 errors and power.

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Matching Cases and Controls Using Genotype Data from a Whole Genome Association Study. *W. Guan, L. Liang, M. Boehnke, G.R. Abecasis.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Whole genome case-control association studies are now being undertaken for many complex diseases such as hypertension, diabetes, and bipolar disorder. Such studies typically require that thousands of samples be genotyped for hundreds of thousands of genetic markers. Interesting markers are those that show substantial allele or haplotype frequency differences between cases and controls. However, population substructure may result in heterogeneity between cases and controls, and can result in spurious disease-marker associations. Approaches exist to match cases and controls prior to genotyping, or based on genotype data for a few genetic markers; such approaches certainly can be useful. However, once genotyping for a whole genome association study is completed, much better case-control matching is possible by using all available genotype data. We propose a strategy for matching cases and controls after whole genome association genotyping is completed based on similarity scores calculated from all available marker genotype data. Through analysis of data from and inspired by the International HapMap project, we demonstrate that our strategy improves case-control matching beyond what is possible prior to whole genome analysis, decreasing false positives and improving power to detect true disease predisposing variants. We further show that our strategy can handle large numbers of samples and markers efficiently. We suggest that initial matching of cases and controls prior to whole genome genotyping combined with careful re-matching after whole genome genotyping is the method of choice for whole genome association studies.

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Calpain 10 and Interleukin 6 variants are associated with polycystic ovary syndrome. *C. Lamina[1], S. Hahn[2], C. Vollmert[1], M. Kolz[1], A. Schöpfer-Wendels[1], K. Mann[2], F. Bongardt[1], J.C. Müller[1], F. Kronenberg[1,3], H.-E. Wichmann[1], C. Herder[4], R. Holle[1], W. König[5], H. Löwel[1], T. Illig[1], O.E. Janssen[2], the KORA group.* 1) GSF-National Research Center, Neuherberg, Germany; 2) University of Duisburg-Essen, Dept. of Medicine, Essen, Germany; 3) Innsbruck Medical University, Innsbruck, Austria; 4) German Diabetes Center, Düsseldorf, Germany; 5) University of Ulm, Medical Center, Ulm, Germany.

Polycystic ovary syndrome (PCOS) is known to be associated with an increased risk of type 2 diabetes mellitus (T2DM) and was proposed to share a common genetic background. Recent studies suggest that Calpain 10 gene (CAPN 10) and Interleukin 6 gene (IL6) variants contribute to genetic predisposition to T2DM and therefore represent strong candidate genes for a role in PCOS susceptibility. We genotyped the CAPN 10 UCSNPs: 44, 43, 56, Ins/Del19, 110, 58, 63, and 22 as well as the IL6 Promotor-SNPs: -598, -573, -174 in 146 German PCOS women (mean age 27 years, BMI 30.2 kg/m2) with 606 age-matched healthy controls (BMI 24.7 kg/m2) taken from a large population based study (KORA study, Augsburg, Germany) using MALDI-TOF MS technique. Genotype distributions were in Hardy-Weinberg equilibrium. Statistical analysis was corrected for age and BMI. An association between CAPN10 and the susceptibility to PCOS was found for UCSNP56 with an odds ratio of 2.91 (CI=1.5-5.6) for genotype AA compared to GG. The 22-allele of the Ins/Del19 variant was also associated with an increased odds ratio for PCOS (2.98, CI=1.55-5.73). For both SNPs, a dose-dependent allele-effect was detected. Eleven CAPN10 haplotypes with frequencies >1% were identified, of which the haplotype TGG3AGCA (frequency 21%, p=0.0057) and the TGA2AGCA haplotype (frequency 4.9%, p=0.0011) were significantly associated with PCOS. For the genotyped IL6 SNPs a significant PCOS predisposition was found in -598_GA/-174_GC-carriers (p=0.0004) compared to -598_AA/-174_CC-allele-carriers. In conclusion, CAPN10 UCSNPs 56 and 19, as well as IL6 SNPs -598 and -174 showed a genotype-dependent association with PCOS, which was confirmed by results of haplotype analysis.

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Association of MAOB Polymorphism and Parkinson's Disease. *S.J. Kang, W.K. Scott, Y. Li, M. Hauser, J.M. van der Walt, K. Fujiwara, J.M. Vance, E.R. Martin.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Parkinson's disease (PD) is a genetically complex disorder that affects more than one million Americans. The inability to control movement in PD patients results from severe loss of dopaminergic neurons within the substantia nigra. The mechanism causing dopaminergic cell death in PD has not been defined although it is generally accepted that environmental and genetic sources both contribute to risk. Monoamine oxidase (MAO) is one of the primary enzymes regulating metabolism of neurotransmitters such as dopamine. Two distinct forms of the enzyme, encoded by genes MAOA and MAOB located on the X chromosome, have been considered as possible factors in the pathogenesis of PD. Previous association studies of PD and MAO genes reported inconsistent results. In this study, we used a large family-based data set to test associations between MAO genes and a risk of PD. The data set includes 298 female discordant sibpairs and 348 male discordant sibpairs. Mean $\pm$ SD age of onset in affected individuals in the sample is 57.85 $\pm$ 13.20 (58.13 $\pm$ 12.70 for males and 57.41 $\pm$13.95 for females). For this study, all subjects analyzed were white and families with known parkin mutations were removed. We analyzed 15 single nucleotide polymorphisms (SNPs) in the MAO genes.

We used the pedigree disequilibrium test (PDT) to test association of the SNPs with PD risk among female discordant sibpairs and male discordant sibpairs separately. We performed the PDT analysis on the sex-specific data sets. Significant association was found with the intron 13 SNP of MAOB in overall female subset (p-value 0.02). No significant association was found in overall male subset. Though only marginally significant, the result is compelling since the same SNP has been found to be associated with PD in previous studies. Our results add to the evidence of involvement of MAOB in PD, and suggest that the effect may be stronger in females.

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Familial Aggregation of Hyperemesis Gravidarum. *M. Schoenberg-Fejzo[1], M. Wilson[1], L. Yedigarova[1], K. Mac Gibbon[2], S. Ingles[1], T.M. Goodwin[1].* 1) USC, Los Angeles, CA; 2) HER Foundation.

Nausea and vomiting or morning sickness during the first trimester of pregnancy affects between 70-90% of gravidas. Pernicious vomiting of pregnancy, or Hyperemesis Gravidarum (HG), is less common, occurring in approximately 0.3-2% of all pregnant women, and is the second leading indicator for antenatal hospitalization. Hyperemesis gravidarum may persist throughout gestation, and the characteristic weight loss of at least 5% in the expectant mother is often considered a defining feature distinguishing HG from typical nausea and vomiting. In addition, the disease can be associated with ketonemia, ketonuria, electrolyte imbalance, dehydration, hepatic and renal damage, and even death. The cause of HG is unknown, however, it is a disease that bridges multiple races, cultures, and species. Significant ethnic differences in the incidence of HG have been reported for multiple populations including higher prevalence in New Zealand pacific island women, United Kingdom Indian and Pakistani women, and African-American women compared with ethnic European women. It is perplexing that nausea and vomiting in pregnancy, a condition that appears to have an ill effect on the health and well-being of the mother and fetus, has not been selected out in nature, nor is it unique to humans. Our analysis of a HER Foundation Survey suggests familial aggregation of HG. 110/628 (18%) sisters with a pregnancy history reportedly share HG. 88/1064 (8%) reported sharing HG with their mothers, and 107/1064 (10%) reported having at least one secondary relative with HG, with 20 reporting a paternal relative, and 22 reporting a maternal relative. 5 sets of twins reportedly shared HG. This provides evidence for a genetic component to HG, making it a disease amenable to genetic studies.

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Complex Segregation Analysis of 1919 Pedigrees with Ovarian Cancer from the Gilda Radner Familial Ovarian Cancer Registry. *B.O. Tayo[1], R.A. DiCioccio[2], Y. Liang[1], M. Trevisan[1], M.S. Piver[3], K. Odunsi[2].* 1) University at Buffalo, Buffalo, NY; 2) Roswell Park Cancer Institute, Buffalo, NY; 3) Sisters of Charity Hospital, Buffalo, NY.

Familial component is estimated to account for up to 10% of ovarian cancer. However, the mode of inheritance of ovarian cancer still remains poorly understood. The goal of this study was to investigate the inheritance model that best fits the observed transmission pattern of ovarian cancer among the 7424 members of 1919 pedigrees ascertained through probands from the Gilda Radner Familial Ovarian Cancer Registry of Roswell Park Cancer Institute, Buffalo, New York. Criteria for proband selection included a family history of two or more cases of ovarian cancer. Using the SEGREG program of the Statistical Analysis for Genetic Epidemiology (S.A.G.E.), we fitted different regressive multivariate logistic models (Mendelian, sporadic, environmental and general) to the data on ovarian cancer status of the 7424 pedigree members. Covariate on affection status for other related cancer sites such as breast, pancreas, uterus/endometrium and prostate, was included in each fitted model. Parameters estimated include the frequency of susceptibility allele, logarithm of the odds of being affected versus unaffected, transmission and transition probabilities for the different models. Our analysis results indicate that Mendelian codominant model provides a significantly better fits than the other models for ovarian cancer.

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An empirical evaluation of tagging SNP strategies for candidate gene association studies. *M. Weale[1], B. Browning[2], K. Ahmadi[3], D.B. Goldstein[4].* 1) Inst. Human Genetics & Health, University College London, London, United Kingdom; 2) GlaxoSmithKline, Research Triangle Park, NC, USA; 3) Department of Biology, UCL, London, UK; 4) Institute of Genome Sciences and Policy, Duke University, NC, USA.

There is currently no consensus on an optimal method for tagging SNP selection (identifying SNPs from a reference sample such as HapMap to be used in a larger association study). Three popular methods are based on different measures of association between the tags and other SNPs in the region: pairwise r2, haplotype r2, and allelic (or locus-scoring) r2. We describe a novel procedure for the empirical evaluation of these methods, and apply it to representative candidate gene regions using HapMap data. In terms of average power, all three methods perform equally well. In terms of genotyping efficiency however (i.e. the number of tagging SNPs required), the two multimarker methods perform considerably better than the pairwise r2 method. We explore the properties of a given genetic region that may predispose it to one or other SNP tagging method.

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An evaluation of power and type I error of single SNP transmission disequilibrium tests under locus heterogeneity. *K.K. Nicodemus, Y. Yao.* Dept Genetic Epidemiology, Johns Hopkins SPH, Baltimore, MD.

The testing of preferential transmission of alleles from parents to affected offspring has become a common method to assess association between genetic markers and disease. The number of variations on the original TDT are a testament to its continued popularity. However, no guidelines exist to assist researchers to decide which method is the most powerful to use, especially under locus heterogeneity. Since TDT-like methods are most often applied to complex disease data, locus heterogeneity may have a strong impact on power to detect association. We evaluated the power and type I error of FBAT, PDT, and TDTPHASE under conditions of 25%, 50%, and 75% of families associated with an observed SNP. Using simulated data with approximately 100 parent-affect child trios, a disease allele frequency of 0.20, and penetrances representing 3 different genetic models (additive: penetrance 1/2=0.25, 2/2=0.5; recessive 2/2=0.50; dominant 1/2 and 2/2=0.50) and 1000 replicates, we found that the association tests gave moderate power to detect association at the presence of locus heterogeneity; power, calculated holding type I error constant at 0.05, for FBAT was superior to TDTPHASE and PDT under all conditions, and the increase in power was from 1.3%-4.0% for TDTPHASE and 1.5%-5.1% for PDT. PDT was more powerful than TDTPHASE in 5 out of 9 simulated conditions. As expected, power increased under the dominant and recessive models versus the additive models. Data was simulated using the same number of families and the same family structure; we also calculated type I error rates. FBAT had a slightly inflated marker-wise type I error of 0.064, whereas both TDTPHASE and PDT showed an almost exactly expected type I error of 0.0498(TDTPHASE) and 0.0499(PDT). All methods showed acceptable levels of type I error although one method (FBAT) was more powerful than the other two methods. We are currently testing the same methods on replicates of 500 families, which is closer to a realistic family-based association study; further, we intend to test other single SNP association methods.

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The determinant of haplotype frequency matrix can evaluate the Linkage Disequilibrium between multi-allelic loci. *R. Takemura[1], S. Kamitsuji[1], N. Kamatani[2].* 1) Japan Biological Information Research Center, JBiC, Tokyo, Japan; 2) Department of Advanced Biomedical Engineering and Science Tokyo Womens Medical University, Tokyo, Japan.

The need for the methods of genome wide linkage disequilibrium (LD) analysis has emerged in order to map common disease genes or the genes associated with drug responses. There are a lot of studies about LD in which pair-wise bi-allelic loci are involved. Thus, D or r2 and other measures reflect the deviation of haplotype frequencies from the state of linkage equilibrium (LE), and thereby indicate the strength of LD. There are few studies about LD in which multi-allelic loci are involved. Zapata et al. (2001) proposed the measure D for LD between multi-allelic loci. However the use of D gives too conservative conclusions, and does not fit the method to discover genes using LD. Then we now propose a new measure for the strength of LD. Imagine that the first locus is composed of m alleles while the second locus is composed of n alleles. The entire haplotype frequencies can be expressed by a m x n matrix whose elements are haplotype frequencies. Our aim is to determine how much the observed haplotype frequencies deviate from expected haplotype frequencies under the assumption of LE. As a simple resolution, we introduce the determinant of matrix. When m=n, Multi Allelic linkage Disequilibrium (MAD) is defined by the determinant of the haplotype frequency matrix. It is noted that MAD can be written as only the determinant of the deviation matrix which is obtained by subtracting haplotype frequency matrix under the assumption of LE from the observed haplotype frequency matrix. Since, however, the range of value of MAD changes by variation of allele frequencies, it may be difficult to evaluate the magnitude of LD by using MAD directly. Then we also propose MAD which standardize MAD so as to take the value from 0 to 1, and the validity of the above method is shown by giving a numerical simulation. In case of mn, MAD is calculated as the square root of determinant of the product between the haplotype frequency matrix and its transpose, and its validity is also shown by giving a numerical simulation.

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Nonlinear tests for genomewide association studies. *J.Y. Zhao[1], L. Jin[3], M.M. Xiong[1, 2].* 1) Human Genetics Ctr, Univ Texas HSC at Houston, Houston, TX; 2) School of Life Science, Fudan University, Shanghai, China; 3) Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH.

As millions of single-nucleotide polymorphisms (SNPs) have been confirmed and high throughput genotyping technologies have been rapidly developed, large-scale genomewide association studies are feasible in practice. Genomewide association studies require performing a large number of multiple tests. After accounting for multiple comparisons, each independent test needs very small p-values to ensure a genomewide significance level of 0.05. Unfortunately, almost no current statistical methods can provide very small p-value for genetic association tests. In this report, we introduce nonlinear tests, which are based on nonlinear transformation of allele or haplotype frequencies, to amplify the differences in allele or haplotype frequencies. We show that a class of similarity measure-based test statistics is based on the quadratic function of allele or haplotype frequencies, thus they belong to nonlinear tests. The type I error rates of the nonlinear tests are validated using extensive simulation studies. Power of the nonlinear test statistics are studies by analytical methods. Power study shows that, in most cases, nonlinear test statistics have higher power than the standard chi-square test statistic. The difference between the power of the nonlinear test statistics and the standard chi-square test statistic depends on the measure of nonlinearity of the nonlinear transformation of allele or haplotype frequencies. To further evaluate the performance of the nonlinear test statistics, they are also applied to three real examples.

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Multipoint linkage analysis for a very dense set of markers. *S. Bacanu.* Genetic Research, GlaxoSmithKline, Research Tr, NC.

Multipoint linkage methods are powerful tools that are often employed as the first means to discover alleles affecting liability to diseases. With the advent of dense marker maps, linkage disequilibrium (LD) between markers is inevitable and it comes at the cost of bias and increased rate of false-positive findings for linkage analyses that assume alleles of different markers are independent. I propose a multipoint on subsets method that avoids this issue by partitioning the markers into interlaced and non-overlapping subsets. Each subset is analyzed separately, their statistics are then averaged, and the resulting average is standardized by its estimated standard deviation. In addition to being robust to the challenges induced by dependent marker alleles, data simulated under linkage equilibrium show that the proposed method does not suffer any detectable loss of power when compared to traditional methods.

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Theoretical comparison of P-value for a disease variant and P-value for the adjacent biallelic marker in case-control association test. *J. Ohashi, K. Tokunaga.* Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Although common disease variants are expected to be identified by fine-scale mapping using case-control association test, a disease variant does not always show smaller P-value than the adjacent polymorphic markers if linkage disequilibrium (LD) between them is strong. It is therefore generally difficult to detect a true disease variant based solely on the P-values for the tested markers being in LD each other. To find a condition that a primary variant associated with the disease can be determined based on P-values from case-control association test, we compared P-value for a disease variant with that for the adjacent biallelic marker being in LD, using a computer simulation analysis where two-locus two-allele model (i.e., one disease variant and one biallelic marker) was assumed. In the simulation, we estimated the probability that P-value for the marker being in LD with the variant is smaller than that for the disease variant for a variety of parameter sets (haplotype frequency, penetrance, sample size). The estimated probability was not so small for small penetrance and strong LD when the sample size is small (e.g., 300 cases and 300 controls). However, P-value for the marker became hardly a one-tenth following of P-value for the disease variant. These results suggest that a single variant primarily associated with the disease can be inferred by case-control association test only when P-value for the variant is markedly smaller than those of all other polymorphisms being in LD with the variant.

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The Khatri Sikh Diabetes Study (SDS): Study Design, Methodology, Sample Collection and Initial Results. *D.K. Sanghera[1], J.S. Bhatti[2], G.K. Bhatti[3], S.K. Ralhan[2], G.S. Wander[2], J.R. Singh[4], C.H. Bunker[5], D.E. Weeks[1], M.I. Kamboh[1], R.E. Ferrell[1].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA; 2) Hero DMC Heart Institute, Ludhiana, Punjab, India; 3) 560-B, Sector 32-A, Chandigarh, India; 4) Guru Nanak Dev University, Amritsar, Punjab, India; 5) Department of Epidemiology, University of Pittsburgh, PA.

Type 2 diabetes (T2DM) has become a major public health problem in India. The pilot or Phase I of the Sikh Diabetes Study (SDS) was launched to build resources to initiate a large scale genetic epidemiological program with an aim to identify the genomic regions responsible for diabetes susceptibility in Khatri Sikhs from North India. With full informed consents and following stringent selection criteria, 1892 subjects were enrolled to participate in this family-based linkage study. Of these, 1623 subjects were recruited from 324 families including 1288 T2DM patients (siblings, parents or relatives) and 335 unaffected siblings, parents or relatives. The remaining 269 subjects were enrolled as unrelated non-diabetic controls. Each one of these families had at least two siblings affected with T2DM with a current total of 515 affected sibling pairs (ASPs). Buffy coats were harvested from EDTA whole blood and DNA was extracted and stored at -80oC. This population has several novel features which are ideal for performing genetic studies. This vegetarian, non-smoking endogamous caste group presents unusual clinical spectrum with relatively younger age of onset and possesses a unique physiology of obesity that remains unexplained by BMI. It is important to study the underlying molecular mechanisms responsible for increased prevalence of T2DM especially in the populations adapted to survival under nutritional thriftiness and physically demanding work. The SDS has created a comprehensive genetic and epidemiological resource. Ours will be the first systematically conducted study to be undertaken in this North Indian population and will facilitate a genome-wide search to map Asian Indian susceptibility genes.

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Incorporation of sex-specific recombination information fails to improve PPL performance. *M.W. Logue, V.J. Vieland.* Center for Statistical Genetics Research, University of Iowa, Iowa City, IA.

The 2-point posterior probability of linkage, or PPL, is a Bayesian statistic which directly measures the probability that a marker is linked to a disease gene. It removes the need to specify the genetic model *a priori* by integrating over the genetic model space. The Bayesian nature of the PPL allows incorporation of prior information, such as the sex-specific recombination ratio ($R=_F/_M$) in the region of the marker in question. In a series of simulations, we explore the effects of assuming a single *sex-averaged* in PPL computation (denoted PPL_{SA}), and compare it to a PPL computed from a *sex-specific* likelihood (as a function of $_M$ and $_F$), denoted PPL_{SS}. We evaluate the PPL_{SS} with two different forms of the prior distribution on ($_M$, $_F$). The first is fixed (independent of R), while the second places additional prior weight based on knowledge of R. We denote the PPLs computed with these priors as the *non-adaptive* (PPL_{SS-NA}), and the *adaptive* (PPL_{SS-A}) sex-specific PPLs. We find that the PPL_{SS-NA} performed almost identically to the PPL_{SA} replicate by replicate, across a large number of generating models. However, the PPL_{SS-A} varied widely from the PPL_{SA} (and PPL_{SS-NA}), being as much as 21% lower, and performed worse, on average, than the PPL_{SA} and PPL_{SS-NA}, when R was large. This is due to considerable variability in the observed likelihood, in that, while maximum likelihood estimates of $_M$ and $_F$ perform well "on average", in any particular replicate peak $_M$, $_F$ locations bear little relation to the true generating value of R, even in very large samples. Thus, even under ideal circumstances, MLEs of $_M$ and $_F$ are not reliable indicators of the true values; and as a result, incorporating prior genomic information is not necessarily helpful in real applications.

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Mixed inheritance model-based method to implement linkage analysis for multiple genetic loci responsible for the complex diseases under threshold model. *A. Narita, A. Tajima, I. Inoue.* Div Genetic Diagnosis, Inst Medical Science, Tokyo, Japan.

As multiple factors, either genetic or environmental, are involved in complex diseases, difficulties in the elucidation of the complicated architecture underlying these diseases are confronting. By incorporating the concept of quantitative trait, we developed an effective tool to detect multiple genetic loci responsible for the complex diseases under the threshold model, in which a discrete trait is actually determined by a normally distributed variable, called liability, that depends on multiple genetic effects (*liability* = + *G* + *e*). In addition, genetic factors affecting predisposition of these diseases are, in many cases, biallelic SNPs (at which one allele increases the risk of affection, whereas the other might reduce). Therefore, instead of the commonly-used variance component model which treats genetic effects as random effects, the mixed inheritance model is adopted. The model considers three possible genotypes at the causative loci as fixed effects and the unobservable genotypes at the loci can be estimated for each individual. At the same time, as the method is based on the Bayesian approach via the Markov chain Monte Carlo (MCMC) algorithm, genotypic effects at each locus and its chromosomal location can be also estimated. Moreover, the reversible jump MCMC algorithm, which is a specialized version of the MCMC algorithm for the determination of the most likely genetic model through iterative additions and deletions of variables, enables estimation of the number of loci responsible for the diseases as an unknown parameter. Effectiveness and flexibility of the method were demonstrated by using simulated data. The results indicate that some potential causative loci, which have an important role in predispositions of these complex diseases but have not been detected previously, would be additionally identified. In the future, loci which have epistatic effects (gene-to-gene interactions) will also be approached.

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A cluster-based SNP linkage mapping set based on genetic distances and on haplotype heterozygosity. *J. Ziegle, F.C.L. Hyland, J. Day, C. Scafe, R. Koehler, N. Peyret, C. Larry, M. Rhodes, T. Woodage, X. You, L. Xu, E. Spier, F.M. de la Vega.* Applied Biosystems, 850 Lincoln Centre Dr., Foster City, CA 94404, USA.

A SNP linkage mapping set that combines the heterozygosity of microsatellite loci and the efficiency, accuracy, cost advantages and automation potential of SNP genotyping is described. To enable high-throughput and cost-effective genotyping, the platform for this human linkage mapping set is the SNPlex Genotyping System, a multiplexed high throughput genotyping platform based on the oligonucleotide ligation assay that utilizes capillary electrophoresis as the readout. A 3.9 cM resolution SNP map for linkage mapping previously developed by The SNP Consortium (TSC) (Matise et al. 2003) was used as the framework. The set is composed of SNP clusters, with spacing between clusters based on genetic rather than physical distance. In the first phase, SNPlex assays for markers in the TSC linkage mapping set were tested. In the second phase, validated SNPs from the TaqMan SNP genotyping assays were chosen to fill gaps between clusters larger than 5 cM. To select new candidate SNPs, NCBI physical coordinates were transformed to genetic distances using the TSC genetic map. The transform was refined using the metric linkage disequilibrium map (expressed in LD Units) derived from the TaqMan assay validation. 10 candidate SNPs were selected for each cluster. Haplotypes were computed for every possible combination of 4 of these 10 SNPs, and the 4 SNPs that maximized haplotype heterozygosity across four populations (Caucasian, African American, Japanese, Chinese) were converted into SNPlex assays using the OLA Probe Designer (OPD) pipeline, and extensively validated to select only highly robust SNPs. A genetic map was calculated using genotypes of 574 samples from 43 CEPH families. Genetic distances proved to be highly correlated with LDU: the average correlation between LDU and observed cM was 0.99. The linkage mapping set is composed of clusters spaced 1.9 cM apart, with the mean distance between SNPs being 1.04 cM. No gaps larger than 10cM are present. The average information content of the set is 0.95.

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A Multimarker Regression-Based Test of Linkage for Affected-Sib-Pairs at Two Linked Loci. *M.J Barber, J.A. Todd, H.J. Cordell.* Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, UK.

We address the analytical problem of evaluating the evidence for linkage at a test locus while taking into account the effect of a known linked disease locus. The method we propose is a multimarker regression approach that models the identity-by-descent states for affected-sib-pairs at a series of linked markers in terms of the identity-by-descent state at the known disease locus. Our method allows analysis to be performed at a test location (or a series of locations) without the requirement that identity-by-descent be directly observed at either the test or the known conditioning locus. An advantage of our method is that identity-by-descent states from multiple markers are included simultaneously in the test of linkage, without recourse to multipoint imputation. The properties and power of the method are examined under various null and alternative hypotheses. The method is applied to data from a type 1 diabetes study to examine the evidence for an additional locus (IDDM15) on chromosome 6, linked to IDDM1 in the HLA region. Analysis in this region is complicated by the strong effect of IDDM1 in type 1 diabetes, and by differing rates of male and female recombination in the region.

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Transmission recoding: a powerful multipoint likelihood algorithm for linkage and linkage disequilibrium mapping. *J.R. O'Connell.* Div Endo/Diabetes/Nutrition, Univ Maryland, Baltimore, MD.

Multipoint likelihood calculations are the core of pedigree-based gene mapping methods. Algorithmic advances continue to extend the range of computationally feasible exact likelihood calculations with regard to both the number of markers and size of pedigrees. Allele consolidation methods such as set recoding as implemented into VITESSE are the most powerful methods to date to speed up the Elston-Stewart algorithm. We introduce a new recoding method called *transmission recoding* based on the simple biological principle that at meiosis only one of two alleles in a parental diploid genotype is transmitted to the offspring while the other allele is lost to genetic drift. When an individual is untyped the allelic state of the non-transmitted allele is unconstrained and only enters the likelihood through its population frequency. Thus, by introducing a universal non-transmitted allele X with unit frequency to represent all alleles at a locus, we can recode genotypes as T/T, T/X and X/X, where T represents a transmitted allele. In addition we introduce a new parent-offspring inheritance rule: a parent genotype T_1/T_2 is consistent with its offspring if and only if *both* alleles T_1 and T_2 are transmitted. Thus, in particular, the only consistent parental genotypes in matings with a single genotyped offspring are of the form T/X, where T is an observed allele in the offspring. Transmission recoding is most powerful in the computationally difficult components of the pedigree containing consecutive generations of missing data where the number of founder alleles lost to genetic drift tends to be large compared to the number of observed non-founder alleles. Timing results show that transmission recoding can speed up the likelihood calculation 2-3 orders of magnitude over set recoding. Moreover, transmission recoding also eliminates biases in maximum likelihood estimates of allele and haplotype frequencies due to imputing unobserved founder alleles and haplotypes, therefore preserving linkage disequilibrium patterns between densely spaced markers. Transmission recoding is a powerful method to improve linkage and association mapping in pedigrees.

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Consequences of exhaustive haplotype association analysis. *B.A. Salisbury, R.S. Judson, M. Pungliya, Y. Zhao, B. Dain.* Genaissance Pharmaceuticals, New Haven, CT.

Purpose: In searching for associations between a phenotype and a genetic locus with multiple polymorphisms, it is possible to test every individual polymorphism and every genetic marker formed by alleles from two or more of those polymorphisms (i.e., haplotypes). We have investigated trade-offs inherent in exhaustively testing markers of ever greater complexity. **Methods:** From each of 11 genes sequenced on 200 subjects we designated two SNPs, one common and one rare, as causal in dominant mode. For each of these 22 SNPs independently, we modeled four continuous phenotypes, two each with raw p-values equal to 0.005 and 0.00005. Associations were sought between each gene and its corresponding phenotypes, with limits of 1 to 5 SNPs per marker. Analyses were run with and without the model SNPs. For each gene-phenotype test, the most significant marker was recorded along with its adjusted p-value, which is the fraction of permuted data sets for which the best marker had a p-value at least as good as the original best. **Results:** Allowing more complex markers decreases raw p-values greatly. When causative SNPs are included in analyses, adjusted p-values get worse with an increasing complexity limit, as one would predict. In contrast, when the causative SNPs are excluded, adjusted p-values improve, on average, as more complex markers are allowed, in some cases rescuing associations that would be lost if testing only single SNPs. Another consequence of allowing more complex markers is that the number of SNPs in the most significant markers goes up, regardless whether the causative SNP is tested. Surprisingly, the number of SNPs in a gene had a negligible effect on adjusted p-values. **Conclusions:** These findings argue for testing multi-SNP markers whenever the true causal sites may not have been included, a possibility that can rarely be excluded. However, resulting associations must be treated cautiously, as the complex markers giving the lowest p-values are likely to be overfit. That larger loci are not inherently more challenging is encouraging and suggests that it may be unnecessary to break them down into small haplotype blocks for analysis.

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Allelic structure of human disease, a simulation study. *M. Kimmel, B. Peng.* Dept Statistics, Rice Univ, Houston, TX.

The success of mapping genes involved in complex diseases using association or linkage disequilibrium based methods depends heavily on the number and frequency of susceptibility alleles of these genes. These methods would be economical and statistical feasible if common diseases are usually influenced by one or a few susceptibility alleles at each locus (CDCV hypothesis), but not so if there is a high degree of allelic heterogeneity. Here, we use population simulations to investigate the impact of various genetic and demographic factors on the allelic spectra of common diseases, and estimate the effective number of alleles of common diseases with in the general human population as well as in isolated populations.

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Sexual dimorphism and large QTL on chromosome 2p influencing serum uric acid levels in hypertensive families from a relative isolate population. *F. Gagnon[1], J. Pintos[2], D. Gaudet[2], J. Tremblay[2], A.W. Cowley[3], P. Hamet[2].* 1) Epidemiology and Comm Med,Univ of Ottawa,Ottawa,Canada; 2) CHUM,Montreal,Canada; 3) Medical College of Wisconsin,Milwaukee,USA.

Epidemiological and experimental data have long suggested a relationship between serum uric acid levels (SUA) and essential hypertension (HT). SUA is ignored in clinical practice because it is believed not to have a causal role in HT. Recently, a biological mechanism by which SUA could cause HT in humans was identified. We completed a genome scan for SUA in 93 families (n=1116), from a French-Canadian relative isolate, ascertained through hypertensive dyslipidemic probands. Using covariated-adjusted (age and sex) joint linkage and segregation analysis based on Bayesian Markov chain Monte Carlo methods, we localized a quantitative trait locus (QTL) with an individual contribution of ~ 13% of total SUA variance. We estimated a strong sexual dimorphism for SUA with an effect of ~22% of the total variance due to being female; age had an effect of ~4%. Multiple regression analysis supported an interaction of age and sex. The SUA QTL is located on chromosome (chr) 2p at the HT locus (OMIM 607329) identified by Angius et al. (2002). We re-analyzed chr 2 adding an interaction term for age and sex to the covariates, which increased by ~2.4 fold the linkage signal, with intensity ratio (IR) for linkage of ~ 26. The IR is the posterior acceptance rate of QTL positions, calculated here for 2 cM intervals, to the prior such rate. We assessed the empirical significance for this QTL using the LOP score (log of the ratio of the posterior probabilities of linkage to the chr 2 QTL and ten simulated chr without QTL)(Daw et al. 2003): LOP 3.13 with an estimated gene effect on the squared-root variance of ~ 31 mol/L. Variance-component analysis of chr 2 provided some additional evidence for this QTL (Lod 1.52). In view of the sexual dimorphism observed for SUA, including our report for higher uric acid heritability estimates in females (Am.J.Hum.Genet 76:815-832, 2005), a model-free linkage analysis adapted for genomic imprinting is under way, as well as genotype by sex interaction variance-component analysis.

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Dissecting Heritabilities for 98 Quantitative Traits in a Large Cohort Study with 6,148 Participants. *W-M. Chen[1], G. Pilia[2], A. Scuteri[3,4], M. Orru[2], G. Albai[2], M. Dei[2], S. Lai[2], L. Usala[2], C. Mameli[2], L. Vacca[2], M. Deiana[2], M. Masala[2], S.S. Najjar[4], A. Terracciano[4], T. Nedorezov[4], A. Sharov[4], A.B. Zonderman[4], P. Costa[4], E.G. Lakatta[4], D. Schlessinger[4], the Progenia Team.* 1) Dept. of Biostatistics, U. of Michigan, Ann arbor; 2) INN-Consiglio Nazionale delle Ricerche, Cagliari, Italy; 3) Unita' Operativa Geriatria, Rome, Italy; 4) Gerontology Research Center, NIA, Baltimore.

Large pedigrees from founder populations offer a powerful setting for genetic studies because of their greater genetic and phenotypic homogeneity. In a cluster of 4 towns in a Sardinian founder population, we recruited a cohort of 6148 participants, comprising over 60% of those aged 14-102. In all participants born within the 4 towns, at least 95% have all grandparents born in the same region. The group includes 4933 sib pairs, 4256 parent-child pairs, 4014 cousin pairs, and 6400 avuncular pairs. Quantitative traits (98) were scored, including cardiovascular risk factors and facets of personality as well as blood tests and anthropometric measurements. Analysis of this large dataset posed novel analytical and computational challenges. Data were fitted under a variety of variance components models incorporating genetic and environmental effects and their interactions. To obtain maximum likelihood estimates of heritabilities of all traits, we implemented a computationally efficient scoring method. Estimates of narrow genetic heritability for cardiovascular and personality traits ranged from about 0.09 to 0.45, while estimates of broad heritability which includes genetic dominance were up to 2-fold higher. For 25 traits, heritability differences were seen between the sexes, with 19 of them more heritable in females. In addition, 48 traits showed differences in heritability between those above and below mean age, with 36 traits more heritable in young individuals. Nevertheless, variance component analyses showed that for nearly every trait, the same QTLs contribute to variation for male and female, young and old. Prospective linkage analyses are predicted to have the power to detect loci that contribute about 10% of genetic variance for single or multiple traits.

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Genome-wide scan of a delayed skin granulomatous reaction in leprosy sibships. *B. Ranque[1], A. Alcaïs[1], N.V. Thuc[2], V.H. Thai[2], E. Schurr[3], L. Abel[1].* 1) unité INSERM 550, faculté Necker, PARIS,France; 2) hospital for Dermato-Venerology, Ho Chi Minh City, Vietnam; 3) Mac Gill university, Montréal, Canada.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae affecting 600,000 people each year. The Mitsuda reaction is a delayed skin granulomatous reaction elicited by intradermal injection of heat killed *M.leprae* that reflects cellular immune response to *M.leprae*. While epidemiological studies have provided evidence for a genetic control of the Mitsuda response, the exact nature of this genetic component remains unknown.

We first conducted a segregation analysis of quantitative Mitsuda reaction in 168 nuclear Vietnamese families ascertained through leprosy patients. Different strategies of analysis provided strong evidence for a recessive major gene controlling the Mitsuda reaction, independently of the leprosy status. Under this genetic model, 12% of the population was homozygous for the recessive allele and predicted to display high values of Mitsuda reaction. Noteworthy, strong residual familial correlations persisted after accounting for the major gene effect.

Subsequently, a genome-wide scan of the quantitative Mitsuda reaction was performed in a subsample of 19 families (110 children), using both model-based (the genetic model being estimated by the segregation analysis), and model-free (quantitative Maximum Likelihood Binomial) approaches. Multipoint analyses provided some evidence for linkage (lod score>1.1) to 5 chromosomal regions, which were further saturated by 30 markers. The model-free fine mapping found a suggestive linkage to the NRAMP1 gene (lod score=2.1), confirming the results of a former candidate gene linkage study. In addition, both model-based and model-free analyses identified a novel chromosomal region. Interestingly, we found a negative correlation between the family-specific lod scores at the two regions, suggesting a negative interaction between them.

So far, this study is the most complete dissection of the genetic control of the Mitsuda reaction, and is expected to cast novel lights into the mechanisms of granuloma formation in leprosy.

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Genomic screening in family-based association testing: a new screening methodology. *A. Murphy[1], C. Lange[1,2].* 1) Biostatistics, Harvard School of Public Hlth, Boston, MA; 2) Channing Laboratory, Harvard Medical School, USA.

Recently, Van Steen et al (2005) introduced a two stage-testing strategy for family-based designs that substantially outperforms standard methodology, e.g. false-discovery rate (FDR, Benjamini and Hochberg 1995), which made genome-wide association testing feasible, even for relatively moderate sample sizes. Their approach is built upon the conditional mean model (Lange et al 2003), and therefore requires variation in the phenotype or trait to be tested for association. However, in most family-based studies, affection status is the primary phenotype of interest. Typically, only affected probands are recruited, making an application of the testing strategy by Van Steen et al (2005) impossible. Here, we propose a completely novel approach that can be applied in such situations to circumvent the multiple testing problem. Our method has the same advantages as the approach by Van Steen et al (2005), but does not require any variation in the phenotype or specification of a phenotypic mean model. We assess power of our screening method by analytical power calculations and simulation studies. Its practical importance is illustrated by an application to a 100K-scan of the Framingham Heart Study.

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Assessing genotyping error rates using test-retest data. *A.D. Anderson.* Department of Statistics and Bioinformatics Research Center North Carolina State Univ, NC.

Estimating genotyping error rates is an important component of quality control when genotypes are to be used in scientific studies (e.g. using phenotype-genotype associations to map complex traits). One common method for assessing the error rate is to evaluate a single genotype many times and calculate the proportion of reported genotypes that were in error. The data we dealt with had an alternate form: A number of genotypes were run twice and we were asked to use the concordance/discordance status of each pair of reported genotypes to estimate the error rate. In this work, we assume a simple model for genotyping error rates and use it to derive a maximum likelihood estimator and associated Wald and score confidence intervals for estimating genotyping error rates from such test-retest data. We then compare the behavior of the two proposed confidence intervals for various sample sizes, genotyping error rates, and types of markers. Our results show that, for small error rates such as might be typically expected in human genetic studies, the score confidence interval is to be preferred. However, when error rates are expected to be high, as might be the case when genotypes are run using degraded DNA (e.g. some forensic analyses), the Wald confidence interval shows better behavior. We conclude with simulation results that demonstrate that our estimator performs well even when our model assumptions are violated.

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Disease association in case-control studies - a latent variable approach. *T. Wang[1,2], H.J. Jacob[2], Z.B. Zeng[3].* 1) Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI; 2) Human Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 3) Bioinformatics Research Center & Department of Statistics, North Carolina State University, Raleigh, NC.

There is a growing utilization of high-resolution genetic markers of single nucleotide polymorphisms (SNPs) in characterization of sequence variants in regions harboring genes that influence common complex traits such as hypertension and diabetes. In this study, we present a method for testing disease association in the case-control based design with multiple tightly linked SNPs. First, an EM algorithm is used to estimate haplotype frequencies in cases and controls separately. Following this, the association between multiple SNPs and a disease is assessed through the existence of a latent trait locus. A likelihood-based statistic provides a comprehensive test for various relevant hypotheses. In case-control association studies, ascertainment of individuals that exhibit a disease phenotype may enrich the underlying disease mutant alleles. Various evolutionary factors may also influence the association pattern in our current sampled populations. Simulation studies are implemented via random coalescent process to evaluate influence of sample ascertainment and various evolutionary factors on the power and asymptotic behavior of the test statistic.

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Likelihood association tests. *T.M. Teslovich, D.J. Cutler.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Conventional genetic disease association test statistics use a relatively small proportion of the available genetic information. Often the test statistic compares the frequency of an allele in Case versus Control individuals, or compares the frequency of transmission of an allele from heterozygous parents to affected offspring (TDT). While these simplified approaches lead to easy-to-calculate test statistics, and are sometimes highly powered, they ignore all the complexity of real disease, such as dominance relationships between alleles, differences in effect in males versus females, and epigenetic (parent of origin) effects. Worse still, these simplified approaches often lead to substantial false positive rates from such real world complicating factors as population stratification in the Case-Control studies and genotyping error in TDT studies. Combining data from these two types of study designs is often impossible in a conventional framework.

Here we describe a new analytical framework, Likelihood Association Tests (LATs) that explicitly accounts for all these real world complications: arbitrary dominance, gender specific effects, epigenetic effects, population stratification, and genotyping error. We demonstrate how to use LATs with either Case-Control or Trio Data, or both simultaneously. We show that in the simplest of situations, LATs are at least as powerful as conventional test statistics (such as TDTs). However, in most situations, particularly involving complex genetic models (gender specific or epigenetic affects), LATs are substantially more powerful than conventional tests. We also show that LATs correctly account for population stratification and genotyping error, and are not subject to false positives from these sources.

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Analysis of the protein polymorphism Tyr402His in the Complement Factor H (CFH) gene in extremely discordant sibpairs with neovascular Age-related Macular Degeneration. *M. DeAngelis[1], I. Kim[1], F. Ji[2], S. Adams[1], A. Harring[1], T. Osentoski[3], A. Capone[3], J. Ott[2], J. Miller[1], T. Dryja[1].* 1) The Ocular Molecular Genetics Institute and the Retina Service, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA; 2) The Laboratory of Statistical Genetics, Rockefeller University, New York, NYInfirmary, Boston, MA; 3) Associated Retinal Consultants, PC., William Beaumont Hospital, Royal Oak, MI.

We studied the Tyr402His protein polymorphism in the CFH gene recently reported by several other groups to be associated with increased risk for all subtypes of age-related macular degeneration (AMD). We ascertained 55 extremely discordant sibpairs in which one member (the index patient) had neovascular AMD and another member (the unaffected sib) had no sign of AMD at the same age or older. Alleles for this variant were detected through direct sequencing. McNemars test was used to determine statistical significance between the His alleles and disease risk. We found a statistically significant association between the Tyr402His protein polymorphism in the CFH gene in the homozygous state only and the neovascular form of AMD (p=.0001 for 2 His alleles vs.presence of 0 or 1 His alleles, 16 informative pairs or p =.03 for 2 His alleles vs. presence of 0 His alleles only for 6 informative pairs). We found no statistically significant deviation from Hardy-Weinberg equilibrium for both affected and unaffected individuals. In calculating an IBS score for this variant, with a heterozygosity of 0.47, observed data revealed that 6 sibpairs shared 0 alleles (expected=3 sibpairs); 22 sibpairs shared 1 one allele(expected=20 sibpairs) and 27 sib pairs were observed to have 2 alleles in common (expected=32 sibpairs). The difference between the observed and expected for 55 sibpairs yielded an 2=1.52, df=2, P =.47. An observed IBS resulted in a score of 1.38 and the expected score was 1.16. Since the expected IBS of 1.16 is lower than the observed IBS score of 1.38 this may suggest that the presence of 1 His allele exerts a modest affect on disease susceptibility to the neovascular form of AMD.

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Association of 3 vitamin D genes with insulin secretion in Hispanic and African Americans: the IRAS Family Study. *C.D. Engelman[1], T.E. Fingerlin[1], C.D. Langefeld[2], A.S. Bagwell[2], S.S Rich[2], R.N. Bergman[3], S.M. Haffner[4], M. Bryer-Ash[5], D.W. Bowden[2], J.M. Norris[1].* 1) University of Colorado at Denver and Health Sciences Center; 2) Wake Forest University School of Medicine; 3) University of Southern California; 4) University of Texas Health Sciences Center; 5) University of California Los Angeles.

Vitamin D is associated with type 2 diabetes and related quantitative traits such as insulin secretion. The vitamin D receptor (VDR) gene, the vitamin D-binding protein (GC) gene, and the vitamin D 1-alpha-hydroxylase (CYP27B1) gene have been implicated, though not consistently, in insulin secretion. All 3 genes are expressed in pancreatic beta cells, which secrete insulin. We genotyped 32 SNPs in the 3 genes (average spacing of 4.5 kb) in 1424 Hispanic American and 604 African American individuals from 90 and 42 extended families, respectively, recruited in the Insulin Resistance Atherosclerosis (IRAS) Family Study. We used the family based association test (FBAT) program to investigate the association of SNPs in the 3 genes and insulin secretion, as measured by acute insulin response (AIR) calculated by minimal model analysis of samples from a frequently-sampled intravenous glucose tolerance test. SNPs were tested individually and in haplotypes containing SNPs associated with AIR and in strong LD with each other (D>.9). In African Americans, 3 contiguous SNPs in the VDR gene, rs9729, rs1544410 and rs2239185, were associated or marginally associated with AIR (p=0.03, 0.07, 0.09, respectively). The AGT haplotype formed by these SNPs was associated with higher AIR (p=0.02). Two contiguous SNPs in the GC gene, rs1491709 and rs4588, were associated or marginally associated with AIR (p=0.03 and 0.06, respectively). The TC haplotype formed by these SNPs was associated with higher AIR (p=0.05). In Hispanics, one SNP in the GC gene, rs222003, was associated with AIR (p=0.02). The CYP27B1 gene was not associated with AIR in either ethnic group. This is the first report of evidence that the VDR and GC genes may play a role in insulin secretion in Hispanic and African Americans, as has been reported in other ethnic groups.

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Case-control statistical comparison of LD patterns for genetic association studies. *D.V. Zaykin*[1], *Z. Meng*[2]. 1) Biostatistics, NIEHS/NIH, Res Triang Prk, NC; 2) Clinical Biostatistics, Merck Research Laboratories.

Characterization of LD as a part of the analysis of haplotypic variation is an important step during association studies. It has been observed that in a region of genetic association the extent of LD is likely to differ between the case and the control groups. Color plots of standardized measures of LD are reported increasingly often. Such plots allow to visually assess the difference in LD between phenotype subgroups and researchers might claim higher LD in the cases based on such assessment. We present approaches for statistical comparison of such pairwise matrices of LD. Our methods avoid the HWE assumption, allow efficient computation, graphical display and statistical characterization of pairwise matrices of LD. Case-control comparison of LD facilitates statistical analysis aimed for identification and description of genetic variation underlying disease susceptibility, efficacy and adverse reactions to drugs.

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Estimation and testing of genotype and haplotype effects in family-based analyses of quantitative traits: comparison of prospective and retrospective approaches. *E. Wheeler, H.J. Cordell.* Department of Medical Genetics, University of Cambridge, UK.

The case/pseudocontrol approach is a convenient framework for family-based association analysis of case-parent trios, incorporating several previously-proposed methods such as the transmission/disequilibrium test and log-linear modelling of parent-of-origin effects. The method allows genotype and haplotype analysis at an arbitrary number of linked and unlinked multiallelic loci, as well as modelling of more complex effects such as epistasis, parent-of-origin effects, maternal genotype and mother-child interaction effects, and gene-environment interactions. Here we extend the method to perform analysis of quantitative as opposed to dichotomous (disease) traits. The resulting method can be thought of as a retrospective approach, modelling genotype given trait value. Application of this method to quantitative traits involves several complications not encountered when analysing disease traits, such as issues related to selected sampling and unmodelled population stratification. Through simulations and analytical derivations, we examine the power and properties of our proposed approach, and compare it to several related methods for single-locus quantitative trait association analysis. All methods are found to give correct type 1 error rates and unbiased parameter estimates when applied to randomly ascertained trios from a single homogeneous population. With randomly ascertained families, in the presence of population stratification, a prospective approach (modelling trait value given genotype) is generally more efficient and interpretable than our retrospective approach. However, our method is found to have some advantages with regard to estimation and interpretability of parameter estimates when applied to selected samples.

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Testing for association based on excess allele sharing in a sample of related cases and controls. *L. Klei, K. Roeder.* Carnegie Mellon Univ, Pittsburgh, PA.

We compare a new matching statistic (MS) to two available methods in which a sample of related cases and controls can be used to determine association signals. MS is based on detecting excess allele matching between all pairs of cases by using the difference between observed and expected IBS for each pair. Expected IBS is determined from the population allele frequencies and the kinship of the pairs. We also explored the Hellinger distance (HD), which is determined by taking the sum of the squared differences of the square root of allele frequencies in cases and controls. These methods were compared to the case control quasi-likelihood score (CC-QLS), which is based on the difference between allele counts in cases and controls, properly adjusted for relatedness (Bourgain et al. 2003). To obtain the estimates for the allele frequencies for these three methods we used BLUE for estimating allele frequencies in complex pedigrees (McPeek et al. 2004). We simulated datasets for a disease with low prevalence (0.02) and moderate to high disease allele frequencies under an additive genetic model. The disease allele was linked to an STR allele using different levels of LD. STRs were simulated using a linkage dataset to mimic naturally occurring markers. We created complex pedigrees with at least 2 related cases. Controls were also drawn from these pedigrees. Our results suggest that HD is more powerful than CC-QLS. We also observe a tendency for HD to be more powerful for low frequency STR alleles, while MS is more powerful for high frequency STR alleles. Our results also suggest that the highest power for HD is found in situations where the allele frequency of the disease matches the frequency of the STR allele. In contrast maximum power of MS is always highest for intermediate STR allele frequency irregardless of the disease allele frequency. Low correlations between MS and HD indicate that the two statistics key in on different characteristics of the linked allele. Therefore, these methods should not be seen as complementary in finding possible association between a disease and a marker for many different situations. We also apply these methods to complex schizophrenia pedigrees.

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Missing Phenotype Data Imputation in Pedigree Data Analysis. *B. Fridley[1], M. de Andrade[2].* 1) Statistics, University of Wiscosin, LaCrosse, WI; 2) Div Biostatistics, Mayo Clinic College of Medicine, Rochester, MN.

Methods to handle missing data have been a statistical area of research for many years. However, little research has been done in the area of missing phenotype information within a pedigree analysis. Recently, Fridley et al (2003) proposed a data augmentation approach within a Markov chain Monte Carlo for imputing missing phenotype information for polygenic models using family data. The imputation for the missing data takes into account the familial relationships and uses the observed familial information. We propose extending the use of data augmentation as a means to impute values for the missing phenotype information, from which a pedigree data analysis using a major gene model could be fit. Using a polygenic model, data augmentation will produce a set of k complete-augmentation data from which a major gene model can be fit. By producing a set of k complete datasets, the total variance associated with an estimate can be partitioned into a within-imputation and a between-imputation component. Data augmentation for missing phenotype information was completed using the Genetic Analysis Workshop (GAW13) data.

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A method for singlepoint qualitative trait mapping in arbitrarily large and complex pedigrees. *M. Abney.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Linkage mapping on large pedigrees is typically more powerful than mapping on smaller pedigrees, given a fixed sample size, yet there are few methods for performing such analyses. Methods that can analyze large pedigrees have been oriented towards either quantitative traits or based on model-dependent, parametric methods. Here, I present a nonparametric method for singlepoint linkage analysis on pedigrees of virtually arbitrary size and complexity. The method is able to compute the allele sharing statistic S-pairs as well as assess significance even in the case of only a single pedigree. The method is fast enough to analyze typical genome screen data. I demonstrate the validity of the test by presenting the results of simulations on a 3,028 person pedigree comprising 13 generations with 50 affected individuals (1,275 pairs). I also examine the consequences of performing an analysis with the pedigree divided into separate subpedigrees.

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A comparison of multilocus mapping approaches in the presence of epistasis and heterogeneity. *J. Tzenova, M. Farrall.* Dept Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics, Univ Oxford, Oxford, United Kingdom.

Several linkage methods have been proposed to assess the evidence that multiple regions contribute to a complex trait. We investigated the performance of five different approaches under two-locus genetic models of epistasis and heterogeneity in samples of affected-sib-pairs (ASP). Two-locus genetic models were specified in terms of the two-locus penetrances, resulting in 9 models of epistasis - four of which fit a multiplicative model, 2 models of heterogeneity, and 2 additive models. Data were also generated under the null hypothesis of neither gene contributing to the trait or a single gene acting under a recessive, dominant, or interference model. Marker genotypes were simulated in samples of 100 ASPs assuming a population prevalence of 10%, sibling recurrence risk ratio of 2, and equal disease allele frequencies at both disease loci. We analyzed each replicate with 1/ Genehunter-two-locus using the two-locus parametric Lod score (Strauch et al., Am J Hum Genet, 2000, 66:1945-1957), 2/ Genehunter-Plus using the epistatic and heterogeneity discrete weighting scheme at each locus (Cox et al., Nat Genet, 1999 21:213-215), 3/ Twoloc - a simultaneous two-locus mapping approach in ASP (Farrall, Genet Epi, 1997, 14:103-117), 4/ GeneFinder - a generalized estimating equation approach (Liang et al., Genet Epi, 2001, 21:105-122), and 5/ simplified logistic regression (Holmans, Hum Hered, 2002, 53:92-102). Significance thresholds were obtained for each method under the four null models and false-positive rates were compared to previously published results where applicable. We evaluated power for each method under the different two-locus models and examined the effect of missing data on power. The results vary according to the two-locus method applied, but overall models of strong epistasis fare well regardless of the mapping approach. Methods which stratify the sample appear to have less power than methods which analyze the unstratified sample across all models. We also investigate parameter estimates obtained from three of the methods under each genetic model in an attempt to obtain a range of parameter values specific to the genetic model simulated.

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Sample Size needed to detect gene-gene interaction with association designs in the presence of genotyping errors.
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The needs of searching for gene-gene interactions and accounting for gene-gene interactions in mapping complex diseases have been well acknowledged by researchers. Gauderman (2002) and Wang and Zhao (2003) have reported extensive studies on the statistical power to detect gene-gene interaction through association studies, but without considering the possibility of genotyping errors. As interest in using single-nucleotide polymorphisms (SNPs) as the markers of choices in gene-mapping studies grows, genotyping errors will unfortunately more and more become an issue, especially for high-throughput genotyping methods. Many researches have been done in mapping genes accounting for genotyping error and have suggested the great impact genotyping error might bring, but no work in detecting gene-gene interaction considering genotyping error has been carried out. Therefore, account for genotyping errors in the detection of gene-gene interactions seems to be a necessary when doing complex disease mapping. In this study, we investigate the sample size needed to detect gene-gene interactions at two disease loci with the presence of genotyping errors for association designs. Logistic regression models for disease risks are applied and different genotyping error models are considered. Our results indicate that the required sample size to detect gene-gene interaction increases as genotyping error rate increases, but the amount increased differs with different error models. The increase for case-control design is much greater than that for case-parent design. With error rate varying from 1% to 5%, the increase in the required sample size can be up to 50% for case-parent design, and 100% for case-control design.

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A Bayesian approach to the analysis of candidate genes association studies. *B. Servin, M. Stephens.* Statistics, University of Washington, Seattle, WA.

We present a new method for the analysis of data on association studies of candidate genes. The experimental design we focus on is as follows: first, a small panel of individuals is resequenced for the candidate genes, to identify SNPs in the gene and to investigate the patterns of Linkage Disequilibrium (LD). Then, based on the panel information, a subset of SNPs (Tag SNPs) that summarize the variation in the gene is selected. Finally a large cohort of individuals (for whom phenotype data and relevant covariates are available) are genotyped at the Tag SNPs only. Current typical analysis of data from this kind of study focus on the Tag SNPs genotype data and phenotype data for the large cohort, and largely ignore the panel data once Tag SNPs have been selected. In particular, many methods focus on haplotype at Tag SNPs to capture indirectly the effect of untyped variants. The method we propose uses the information on the panel and avoid relying on indirect associations with Tag SNPs. In order to achieve this we proceed in two steps. First, using models for LD in the gene, we estimate the rates of recombination within the gene, the haplotypic phase, and the genotype data at all untyped (ie non-Tag) SNPs. In doing this, we aim to exploit the fact that Tag SNPs are usually selected in such a way that their genotypes are good predictors of the genotypes at non-Tag SNPs. Second, we use a Bayesian regression model to estimate the posterior probability that each SNP is responsible for phenotypic variation. We do this in a Bayesian framework by iterating the two steps outlined above. The first advantage of this setting is that it provides a natural framework within which to account for the fact that the imputed genotypes are only estimates of the actual genotypes. The second advantage is that, in our view, Bayesian approaches to regression can be designed to cope much more satisfactorily with highly correlated covariates (SNP genotypes) than a standard regression approach. We illustrate our method for a simulated case / control study and for a real study on a quantitative trait.

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Tagging strategies for the design of complex disease association studies: evidence from large-scale empirical and simulated datasets. *E. Zeggini[1], W. Rayner[1], A. Morris[1], G.A. Hitman[2], M. Walker[3], A.T. Hattersley[4], P. Deloukas[5], L. Cardon[1], M.I. McCarthy[1], on behalf of the International Type 2 Diabetes 1q Consortium.* 1) WTCHG, University of Oxford, Oxford, UK; 2) Centre of Diabetes and Metabolic Medicine, Barts and The London Queen Marys School of Medicine and Dentistry, London, UK; 3) School of Clinical Medical Sciences, University of Newcastle, Newcastle, UK; 4) Centre for Molecular Genetics, Peninsular Medical School, Exeter, UK; 5) Wellcome Trust Sanger Institute, Hinxton, UK.

To evaluate the performance of alternative strategies for defining sets of tag-SNPs on the capacity to capture common and rare variation, we examined large-scale empirical and simulated datasets. As part of the International T2D 1q Consortiums efforts to positionally clone T2D susceptibility variant(s) on 1q, a dense map of SNPs (3000 over 13.5 Mb, the majority from the HapMap but with a proportion of SNPs with MAF<5%) has been genotyped in over 4000 individuals from 7 populations. To ensure that findings were not compromised by the inevitable biases in SNP ascertainment, we repeated studies in simulated datasets generated under the neutral coalescent model. In both datasets, we assessed the effects of training set sample size, MAF distribution and tag-SNP selection method on the proportion of common (MAF>5%) and rare (MAF 1-5%) variation captured in test data. We find that >80% of common SNPs are captured (r^2>0.8) with training-set sample sizes over 45 individuals, irrespective of the tag-SNP selection method used: performance does not improve substantially with larger samples. Tagging of rare SNPs is consistently poor irrespective of training set sample size when tag-SNPs are restricted to common SNPs only (though aggressive analyses outperform pairwise methods). The power to tag rare variants improves when the tag-set is allowed to include SNPs of any MAF, showing a linear increase with training set sample size and reaching 80% of rare variants for training-set sample sizes over 100. This increase in performance, however, necessitates that most of the rare variants be included in the tag-set, at the price of a substantial increase in the number of tags that have to be genotyped.

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A full pedigree based method to detect genetic anticipation with application to Huntington's Disease and Li-Fraumeni Syndrome. *T.J. Costello[1], G.E. Bonney[2], L.C. Strong[3], C.I. Amos[1].* 1) Epidemiology, MD Anderson Cancer Center, Houston, TX; 2) Statistical Genetics and Bioinformatics, Howard University, Washington, DC; 3) Clinical Cancer Genetics, MD Anderson Cancer Center, Houston, TX.

Genetic anticipation is defined as a decrease in age of onset or increase in severity as a disease is passed through subsequent generations. Anticipation affects several diseases including Huntington's Disease, Myotonic Dystrophy and Fragile X Syndrome. Additionally, it is suspected in numerous mental disorders (e.g. Schizophrenia, Bipolar Disorder), cancers (Li-Fraumeni Syndrome, Leukemia) and other complex diseases. Several statistical methods have been applied to determine whether anticipation affects a particular disorder, beginning with standard statistical tests and continuing through the development of an affected parent/affected child pair method by Huang and Vieland (1997). These methods have severe limitations for assessing anticipation for a variety of reasons, including familial correlation and low power. Tsai et al (2005) describes a new parent-child pair method that accounts for correlated data in nuclear families, but the authors note that some parent-child pairs may still have to be discarded. Therefore, we have developed a likelihood-based method to assess anticipation in families that appropriately models the underlying transmission of the disease gene and penetrance function. We will present results from simulation studies comparing data sets with differing levels of anticipation versus no anticipation, genetic heterogeneity and missing data along with type I error rates of the method. Preliminary results from simulations suggest this method has good power to detect anticipation and the application of this method to Huntington's Disease and Li-Fraumeni Syndrome data sets revealed evidence for a generation effect in both cases.

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Joint analysis of false discovery rate and non-discovery rate. *L. Sun*[1,2,3], *R.V. Craiu*[2]. 1) Department of Public Health Sciences, Univerity of Toronto; 2) Department of Statistics, University of Toronto; 3) Hospital for Sick Children Research Institute, Toronto.

The multiplicity problem has become increasingly important in genetic studies. The seminal work of Benjamini and Hochberg (1995) on the control of the false discovery rate (FDR) has brought forth a way of thinking about multiple hypothesis testing that is often more relevant than the one based on controlling the family-wise error rate (FWER). In the context of FDR, we propose and study a new quantity, the fraction of non-rejections among the cases in which the null hypotheses are false, which we call non-discovery rate (NDR). We argue that, when a large number of hypotheses are tested simultaneously, NDR could be naturally interpreted as type II error rate if FDR is considered as type I error rate, and we show that much can be gained from a clear understanding and representation of the dependence between FDR and NDR. In particular, we are concerned with the trade off between FDR and NDR and its impact on the choice of FDR and sample-size calculation for a desired NDR. The ideas are illustrated using a series of real-data examples, including microarray data and pedigree data.

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Association analysis with phenotypic covariates using MDR-Phenomics. *H. Mei[1], ML. Cuccaro[2], MA. Pericak-Vance[2], ER. Martin[2].* 1) Bioinformatics Research Center, North Carolina State Univ, Raleigh, NC; 2) Center for Human Genetics, Duke Univ, Durham, NC.

Mapping genes for complex diseases is hindered by genetic heterogeneity. Many common complex disorders are characterized by phenotypic variation, which can be used to harness genetic heterogeneity. Traditional approaches for incorporating phenotypic covariates rely on stratified analysis, which can lose power when there are many possible strata with no a priori knowledge of optimal groupings. We present a novel statistical approach, MDR-Phenomics (MDR-P), developed to jointly integrate discrete phenotypic covariates with genetic data to identify associations with disease. MDR-P is based on multifactor dimensionality reduction (MDR). It uses the genotype-pedigree disequilibrium test statistic and a modified permutation test (Extended-MDR; Mei et al 2005) to look for significant association in family triads. MDR-P classifies genotypes into high-risk and low-risk groups based on combinations of levels of phenotypic covariates and genotypes. To evaluate MDR-P, we used the simulation program SIMLA to model different levels of heterogeneity and phenotypic covariates. We compared power and type I error for three methods: analysis of the overall dataset without stratification by the phenotypic covariate (NS); stratification by phenotypic covariate with the Bonferroni adjustment (SBA); and MDR-P analysis. We found that all methods have similar type I error with estimates equal to or below the nominal level. In general when the phenotypic covariate was restricted to two levels, SBA was more powerful than MDR-P; both exceeded NS. As the number of phenotypic levels increase, MDR-P becomes more powerful than the SBA approach. This study shows that MDR-P is a valid approach and better able to detect disease genes in the presence of heterogeneity than stratified analysis. Like the MDR, MDR-P is an efficient procedure for dealing with large numbers of phenotypic covariates and genetic loci without substantial loss of power. Further, the patterns of association identified through MDR-P can give clues regarding phenotypic attributes important in defining more homogeneous subgroups.

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A 2-locus TDT: properties in the presence of epistasis and population stratification. *S. Kotti, M. Bourgey, F. Clerget-Darpoux.* Inserm U535, Paris, France.

Over the last few years, there has been great interest in assessing the interactive effects of genes because of the important role they may play in the etiology of most human diseases. Until now, several gene-gene interactions have been reported in multifactorial diseases but mainly when one gene effect has already been evidenced. Methods, such as the Conditional TDT, have been proposed to test conditionally to the first gene, the effect of a second one and their interaction. However, it is important to note that this method cannot be applied anymore in the case of epistatic models with weak or no marginal effect at each of the susceptibility loci.

As an alternative, we developed a new method: the 2-locus TDT which has the advantage to be used under such a situation. Our approach is applicable to nuclear family data genotyped for two candidate genes and compares the conditional genotype of the offspring (case genotype) formed by the transmitted gametes given parental genotypes versus the genotype (internal control genotype) formed by the non transmitted gametes.

Using formal computation, we compare our method with the Conditional TDT in terms of:

(i) robustness to population stratification,

(ii) power under different epistatic models.

We show that, unlike the Conditional TDT, the 2-locus TDT is robust to population stratification. In addition, even under no population stratification, the 2-locus TDT may be more powerful than the Conditional TDT.

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REGENERATOR: an analysis tool for genetic association testing in extended pedigrees and genealogies of arbitrary size. *K. Allen-Brady, J. Wong, N.J. Camp.* Genetic Epidemiology, Department of Medical Informatics, University of Utah, Salt Lake City, UT.

We present a general approach to testing for association and transmission-disequilibrium of genetic markers and qualitative or quantitative traits in pedigrees and genealogies of arbitrary size and structure. Our approach uses Monte Carlo significance testing to provide a valid test for related individuals that can be applied to any test statistic, and is not limited to statistics with a known distribution. The REGENERATOR software we introduce incorporates several standard statistical tests for association, including chi-square, trend test, odds ratio, means test, ANOVA, as well as several transmission disequilibrium statistics for qualitative and quantitative data. We illustrate the method and software by analyzing breast cancer and age-at-diagnosis data for associations with a tagging-single nucleotide polymorphism (tSNP) in the ATM gene in high-risk Utah breast cancer families. No association with breast cancer was observed. However, a significant difference (p=0.038) was seen for age-at-diagnosis of breast cancer (mean age SD) between heterozygous carriers of the rare allele (54.2 12.3 yrs) compared to the wild type genotype (57.2 13.7 yrs). This reduction in diagnosis age was due mostly to an increase in the percentage of individuals classified as heterozygous being diagnosed < 50 years old compared to individuals with the wild type genotype, and more individuals with the wild type genotype being diagnosed at > 70 years. We conclude that this tSNP has a small but significant effect on age-at-diagnosis of breast cancer.

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Population-based Methods For Association Mapping Of Quantitative Traits: Model-Free Approaches. *S. Ghosh, A. Mondal.* Human Genetics Unit, Indian Statistical Inst, Kolkata, India.

Although statistical methods for association mapping of binary traits have been extensively proposed and compared, development of such methods for quantitative traits is currently an active area of interest. The paradigm of association for quantitative traits is not very obvious and methods have generally considered the intuitive concept of differences in allele frequencies between individuals with high values of the quantitative trait and those with low values of the trait as evidence of linkage disequilibrium. While some novel methods of association (Allison 1997, George et al. 1999, Abecassis et al. 2000) for quantitative traits in family-based studies have been developed, population-based quantitative data have usually been analyzed using classical analysis of variance (ANOVA) methods. However, ANOVA is valid in a strict statistical sense only under the assumption of equality of variances in each underlying group. On the other hand, the assumption of equality of variances of the quantitative traits at the different QTL genotypes is genetically unrealistic. Thus, it is of interest to explore for model-free alternatives which would circumvent this problem. We propose two methods: (i) a quantile-based regression and (ii) a goodness of fit chi-square to test for allelic association. The basic paradigm of both the methods is that a marker allele in linkage disequibrium with an allele at the QTL would have a non-uniform frequency distribution across the range of quantitative trait values. We perform Monte-Carlo simulations for different genetic parameters to assess the powers of our proposed methods and find that the two methods give comparable powers.

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Statistical Methods for Detecting Microdeletions. *C.C. Wu, S. Shete, C.I. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

Small chromosomal deletions, which have been demonstrated as a fairly common cause for several Mendelian diseases such as Prader-Willi syndrome and Velo-Cardio-Facial syndrome, are commonly observed in children with mental retardation and have been implicated as causally related to some complex diseases, such as autism and schizophrenia. Microdeletions range in size from < 1kb to 4Mb. The density of available microsatellites is only sufficient to detect large deletions. By using single nucleotide polymorphisms (SNPs), the high density of genetic maps would, in principle, be sufficient to identify small deletions. However, microdeletions at microsatellites are more readily detectable than identified from SNPs because of higher information content. We have developed methods for detecting microdeletions using a case-control approach to identifying regions showing an excess of homozygous marker strings in affected individuals. At microsatellite markers, we proposed statistical tests based on binomial distributions and combinatorial models. We suggested to apply the scan statistic to SNP markers. We are evaluating our methods on the data from studies of rheumatoid arthritis and also on simulated data.

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The Restricted Partition Method used to screen for genetic interactions. *R. Culverhouse, B. Steinmeyer, W. Shannon.* Washington University School of Medicine, St. Louis, MO.

Epistatic interactions play an important role in complex diseases: a few loci with little or no main effects can nonetheless control phenotype to a large degree. The Restricted Partition Method (RPM) was developed to examine multi-locus genotypes as predictors of quantitative traits in unrelated individuals. Earlier work verified three important properties: (1) the RPM is robust to allele frequency variations in both contributing loci and null loci, (2) the RPM is computationally feasible and has good power for the analysis of 3-locus models, and (3) two-locus analyses of 3-locus models can have good power to identify contributing loci.

Data from the Pharmacogenetics Research Network Analysis Workshop II consists of real genotypes from which phenotypes were simulated. This data provided an opportunity to test the RPM in three ways: (1) qualitative as well as quantitative phenotypes, (2) a greater number of markers than the RPM was originally intended to handle, and (3) a realistic pattern of linkage disequilibrium.

The RPM was used to scan for univariate effects and 2-way interactions among 4 environmental covariates and 133 candidate SNPs from a sample of 557 unrelated individuals. Phenotype and covariate information was complete, but a sizable number of genotypes were missing. The RPM correctly detected a significant gene-environment interaction related to a qualitative phenotype. In addition, a region of high linkage disequilibrium where multiple SNPs were significantly associated with a quantitative phenotype was identified. The causative SNP could clearly be identified from the analysis. The use of a single replicate data set was sufficient to eliminate the false positives from both of these initial analyses.

Key Results: Designed to detect interactions contributing to a quantitative trait, the RPM performed well in an analysis of a qualitative trait. Further, when faced with a single causative locus in LD with surrounding loci, the RPM correctly identified a univariate model over an interaction model.

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Investigating gene-gene interactions in breast cancer suscpetibility. *P. Pharoah[1], J. Tyrer[1], M. Ostergaard[2], A. Cebrian[1], A. Dunning[1], D. Easton[2], B. Ponder[1].* 1) Oncology, Univ Cambridge, Cambridge, United Kingdom; 2) Public Health and Primary Care, Univ Cambridge, Cambridge, UK.

Much research has been aimed at finding common genetic variants associated with disease, mostly concentrating on the role of individual variants (SNPs). There is a large body of research into theoretical approaches to the study of gene-gene interactions, but the few studies using real data have been limited to data sets with a small number of SNPs. We have previously genotyped 52 tagging SNPs in 10 candidate genes from the antioxidant defence pathway in ~2,200 breast cancer cases and 2,200 controls. The data showed borderline evidence for a main effect for two SNPs. The aim of this analysis is to identify interactions between any of these SNPs in the presence or absence of a measurable main effect. If the form of the interaction were predictable, methods could be tailored to maximise power to detect them. However, the likely models for important interactions are not known, and so we have implemented four different analytic approaches. These are: A) Hierarchical clustering which groups the study subjects independent of case-control status using all the data for classification. Case control frequency in the groups is then compared. The remaining methods search for important combinations of two, three or possibly four SNPs. B) Multifactor dimensionality reduction (MDR) searches for SNP subsets which best discriminate between cases and controls. C) Full interaction models use a saturated logistic likelihood model to search for SNP subsets which give the largest significance value when compared with a null hypothesis of no interaction. D) A single cell search which identifies the most significant combinations of SNP genotypes that vary in frequeny between cases and controls. These methods have been applied to the data described above. As the number of possible interactions is very large the significance of any positive findings will be assessed using permutation testing. We shall present the results and discuss their implications particularly in the context of the possibility of searching for interactions between larger number of SNPs e.g. in a whole genome association study.

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Risk Factor interactions and genetic effects associated with post-operative atrial fibrillation. *A.A Motsinger[1], B.S. Donahue[2], N.J. Brown[3], D.M Roden[3], M.D. Ritchie[1].* 1) Center Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN; 3) Department of Medicine, Vanderbilt University Medical Center, Nashville, TN.

Postoperative Atrial Fibrillation (PoAF) is the most common arrhythmia after heart surgery, and continues to be a major cause of morbidity. Due to the complexity of this condition, many genes and/or environmental factors may play a role in susceptibility. Previous finding have shown several clinical and genetic risk factors for the development of PoAF. The goal of this study was to determine whether interactions among candidate genes and a variety of clinical factors are associated with PoAF. We applied the Multifactor Dimensionality Reduction (MDR) method to detect interactions in a sample of 940 adult subjects undergoing elective procedures of the heart or great vessels, requiring general anesthesia and sternotomy or thoracotomy, where 255 developed PoAF. We took a random sample of controls matched to the 255 AF cases for a total sample size of 510 individuals. MDR is a powerful new statistical approach used to detect gene-gene or gene-environment interactions in the absence of main effects. We chose polymorphisms in 6 candidate genes, all previously indicated in PoAF risk, and a variety of environmental factors to analyze. We detected an interesting single locus effect of IL-6 which is able to correctly predict disease status with 59.8% ($p<0.001$) accuracy. We also detected an interaction between history of AF and length of hospital stay that predicted disease status with 68.34% ($p<0.001$) accuracy. These findings demonstrate the utility of novel computational approaches for the detection of disease susceptibility genes. While each of these results looks interesting, they only explain part of the PoAF story. It will be important to collect a larger set of candidate genes and environmental factors to better characterize the development of PoAF. Applying this powerful new approach, we were able to elucidate potential associations with postoperative atrial fibrillation.

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MC4R variant is associated with measures of adiposity in the general population. *M.R. Lee[1], S.B. Manuck[2], R.E. Ferrell[1].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Psychology, Univ Pittsburgh, Pittsburgh, PA.

Background: Rare missense and nonsense mutations in the melanocortin-4 receptor (MC4R) gene are a cause of genetic forms of severe obesity, and targeted disruption of the mouse MC4R leads to obesity. The role of variation at the MC4R locus in influencing interindividual variation in body size and composition in the general population is controversial. **Objective**: To test the hypothesis that polymorphic variation at the MC4R locus is significantly associated with measures of adiposity in the general population. **Methods**: A single nucleotide polymorphism, -4599 G>T, in the 5-flanking region of MC4R was verified by resequencing in 16 individuals and genotyped in the larger sample by fluorescence polarization. 810 healthy, non-Hispanic white volunteers, age 30-54 years, were recruited from the Pittsburgh community. A medical and demographic history was collected and anthropomorphic measures, fasting lipoproteins, glucose and insulin and resting metabolic rate were measured. ANOVA was used to assess the relationship between genotype and metabolic parameters. **Results**: BMI was greater in participants having a G allele (GG + GT genotypes) than among TT homozygotes ($p < 0.02$), and this association was of similar magnitude in both men (BMI 28.2 vs. 27.5) and women (BMI 26.9 vs. 25.8). Nominally defined overweight (BMI 27) also varied significantly (Chi-sqare = 7.289, $p < 0.03$) across MC4R genotypes (GG: 53.9%; GT: 47.3%, TT: 39.9%). Finally, subjects with any G allele had significantly higher percent of body fat (29.9% vs. 28.8%; $p < 0.05$) and greater waist circumference (36.4 vs. 35.6; $p < 0.03$). Total, LDL-, HDL-cholesterol, fasting insulin, glucose and HOMA and resting metabolic rate were not associated with MC4R genotype. **Conclusion**: Common variation in the 5-flanking region of the MC4R gene is significantly associated with measures of adiposity in men and women in the general population, but is not associated with other indicators of the metabolic syndrome.

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Functional Polymorphisms of the CYP1B1 Gene Predict the Onset of Natural Menopause in Chinese Females.

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Menarche and menopause are among the significant milestones in a female's life. Ages of menarche and menopause and overall menstruation years may influence the health in later life. The Cytochrome P450 1B1 (CYP1B1) plays an important role in mediating estrogen metabolizing. Relatively little is known, however, about the impact of this gene on the onset and cessation of menstruation. In this study, three functional SNPs, including Arg48Gly, Ala119Ser, and Leu432Val, were genotyped in 1227 healthy Chinese females. Among them, 387 women experienced natural menopause. There was no evidence of relationship between the CYP1B1 gene and menarcheal age; however, we did find significant associations for menopausal age and overall menstruation years among natural menopause women, with P values of 0.015 and 0.017 for Arg48Gly, 0.013 and 0.010 for Ala119Ser, and 0.002 and 0.006 for Leu432Val, respectively. The Gly and Ser alleles of Arg48Gly and Ala119Ser were associated with later menopause and longer menstruation years, while, women with allele Val at Leu432Val had a 1.6 yrs earlier menopause and 1.6 yrs shorter reproductive span. Similar significant associations were observed in the haplotype analyses, with P values of 0.026 and 0.022 for menopausal age and menstruation years, respectively. Furthermore, we ascertained the correlations of several personal parameters with menopausal age, such as education level (P=0.010), physical activity (P<0.001), number of live birth (P=0.048) and history of miscarriage (P=0.050). In conclusion, the functional CYP1B1 SNPs and some lifestyle and reproductive parameters may predict natural menopausal age in Chinese females.

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Associations of repair gene polymorphisms and genotoxicity of pregnant women exposed to smoking hazard. *C.J. Chan[1], T.C. Lai[3], C.C. Lee[4], L.Y. Lin[5], T.H. Chiu[4], J.C. Ying[6], H.L. Yang[2], H.D. Wu[1], F.Y. Wu[1].* 1) Institute of Environmental Health, China Medical University, Taichung, Taiwan; 2) Institute of Nutrition, China Medical University, Taichung, Taiwan; 3) Department of Medical Technology, Chungtai Institute of Health Sciences and Technology, Taichung, Taiwan; 4) Department of Obstetric and Gynecology, China Medical University Hospital, Taichung, Taiwan; 5) Institute of Medical Science, Chung Shan Medical University, Taichung, Taiwan; 6) Department of Healthcare Administration, I-Shou University,Kaohsiung, Taiwan.

The purposes of this study were to examine the possible effect of cigarette smoking or environmental tobacco smoke (ETS) on the health of pregnant women, by measuring cotinine concentrations and comet assay, and to analyze the correlations of repair gene polymorphisms and genotoxicity of gestation women. With consented, pregnant women participated prenatal care at two medical centers and one regional hospital in central Taiwan were recruited in this study. The subjects were asked to complete a structured questionnaire. Urine and serum cotinine level were measured using HPLC/UV. PCR-RFLP method was utilized to determine XRCC1, XRCC3, XPD and hMLH1 genotypes. DNA damage was measured by comet assay. The 237 pregnant participants included 102 (43%) non-smokers, 118 (49.8%) passive smokers, and 17 (7.2%) smokers. The findings indicated that the cotinine levels in serum and urine collected at deliver elevated significantly as the exposure to ETS and smoking increased. Our data suggest that the comet assay is a useful tool for detecting DNA damage, and the level of DNA damage among the smoking and ETS groups were significantly higher than that of non-smoking group. The value of DNA damage among women with hMLH1 A/A or G/A genotypes was significantly higher than in subjects with G/G genotype. (aOR=3.49; 95%CI: 1.32-9.24). Genotypic variants of these polymorphisms did elevate DNA damage for women exposed to smoking hazard, especially in ETS group (p=0.021). In conclusion, hMLH1 A/A or G/A genotypes may modify the correlations between Smoking or ETS-exposed and DNA damage.

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The relationship among birth outcome, cotinine level and CYP2A6 polymorphism of neonates in ETS-exposed pregnancy maternity. *H.C. Tsui[1], R.Y. Wang[1], C.C. Lee[2], L.Y. Lin[3], T.H. Chiu[2], H.D. Wu[1], H.W. Kuo[1], F.Y. Wu[1], J.S. Lai[1].* 1) Institute of Environmental Health, China Medical University, Taichung, Taiwan; 2) Department of Obstetric and Gynecology, China Medical University Hospital, Taichung, Taiwan; 3) Institute of Medical Science, Chung Shan Medical University, Taichung, Taiwan.

Previous studies have shown that cigarette smoking contributed to various harmful effects for pregnancy maternity and neonates, and cotinine level may be an important indicator of the metabolic process. On the other hand, CYP2A6 is a metabolic enzyme for nicotine to cotinine. The purpose of this study was to evaluate the correlations among infants CYP2A6 genetic variants, cotinine levels and birth outcomes (including height, weight, head circumference etc.) for environmental tobacco smoke (ETS) exposed and non-exposed. We recruited 227 paired samples of pregnancy maternity and newborns from gynecology outpatient department of three hospitals in central Taiwan. The blood specimens, neonatal birth outcomes, and structured questionnaire data were collected. Serum cotinine level was determined and CYP2A6 was genotyped. The 227 participants included 121 (53.3%) ETS-exposed and 106 (46.7%) non-ETS exposed individuals. Maternal occupation (P=0.0009) and education level (P=0.0005) were significant variables between the two groups. There was significant difference between two groups in infants head circumference (HC) (p=0.0167). The cotinine levels between maternal and neonatal showed highly significant correlation (r=0.88, p<0.0001). Our data revealed that infants with CYP2A6*4C/4C genotype had low cotinine levels compared with those of CYP2A6*1A/4C and CYP2A6*1B/4C (p=0.0365 and p=0.0399, respectively). The HC of neonates with CYP2A6 *4C/4C genotype were smaller in ETS than Non-ETS group (33.81.28 cm vs 32.91.65 cm, p=0.0396). In conclusion, our results suggested that CYP2A6*4C/4C variant in neonates born from ETS-exposure maternities correlated to HC of neonates. In the future, extensive studies to identify the effects of other related genes and environmental factors in neonatal birth outcomes will be warranted.

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Environmental tobacco smoke, metabolic gene polymorphisms, genotoxicity and pregnancy outcome. *F.Y. Wu[1], H.D. Wu[1], H.L. Yang[2], L.Y. Lin[5], C.C. Lee[4], C.J. Lin[3], T.H. Chiu[4], H.W. Kuo[1], J.S. Lai[1].* 1) Institute of Environmental Health, China Medical University, Taichung, Taiwan; 2) Institute of Nutrition, China Medical University, Taichung, Taiwan; 3) Department of Nursing, China Medical University, Taichung, Taiwan; 4) Department of Obstetric and Gynecology, China Medical University Hospital, Taichung, Taiwan; 5) Institute of Medical Science, Chung Shan Medical University, Taichung, Taiwan.

The purposes of this study were to examine the effects of cigarette smoking or environmental tobacco smoke (ETS) on the health of pregnant women and their neonates, and to investigate the inter-relationship among metabolic gene polymorphisms, genotoxicity and pregnancy outcome of gestation women exposed to smoking hazard. Pregnant women sought prenatal care at two medical centers and one regional hospital in central Taiwan were invited to participate in the research as study subjects voluntarily. Six hundred and eighty-five subjects completed the initial questionnaire survey. Metabolic gene polymorphisms of 421 mothers were analyzed. Eventually, 398 subjects remained at labor, and all of them delivered live birth singleton (384 newborns). Comet assay was measured from lymphocytes 291 mothers. Our finding indicated that the levels of DNA damage among the smoking and ETS groups were significantly higher than that of non-smoking group, especially for the ETS group. However, it was not related to metabolic polymorphisms genes. In addition, the results revealed that the average birth weight of neonates born to subjects with severe DNA damage (within 90th percentile, DNA damage score greater than 129.5) was 141g lighter than that of their counterparts (p=0.09). The risk of DNA damage for participants who exposed to EST at home was 4.35 times greater than their counterparts (aOR =4.35). In conclusion, Our study verified the negative relationship between high level of DNA damage among pregnant women and low birth weight (LBW). Pregnant women exposed to ETS were at higher risk of DNA damage than smokers, but they did not aware of the hazard.

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Prostate Cancer, sun expsoure and genes modifying risk. *J. Dickinson[1], T. Dwyer[2,1], L. Blizzard[1], J. Fryer[1], B. Patterson[1], A. Pollanowski[1], J. Stankovich[1,3], J. McKay[1,4].* 1) Menzies Research Institute, Univ Tasmania, Hobart, Tasmania, Australia; 2) Murdoch Children's Research Institute, Melbourne, Victoria; 3) Walter and Eliza Hall Institute, Melbourne, Victoria; 4) International Agency for Research on Cancer, Lyon, France.

UVR exposure may have a protective effect in a number of diseases including prostate cancer. Furthermore, circumstantial evidence exists suggesting that this effect may be mediated by vitamin D status and/or vitamin D receptor polymorphisms, although results to date are unclear. Evidence is also mounting that the selected genes playing pivotal roles in the physiological response to the UVR exposure also have a role in modifying cancer risk. The Tasmanian Prostate Cancer Case Control Study is currently being conducted in Tasmania, Australia. Cases under the age of 70 years identified from the Tasmanian Cancer Registry and age-matched disease free controls selected at random from the Tasmanian Electoral Roll are currently being recruited to the study, recruitment target 400 cases and 400 controls. Comprehensive data comprising family history, dietary factors, physical activity, current and past history of sun exposure is being collected together with vitamin D levels. In data to hand, odds of low (50 mmol/L) vitamin D were 4.66 (95% confidence interval 1.70-12.74) times higher for cases than controls among those with vitamin D measured in higher-UVR months, and 1.22 (0.58-2.56) times higher among those with vitamin D measured in lower-UVR months.The odds of low (50 mmol/L) vitamin D were 2.14 (1.21-3.78) times higher for cases than controls when adjusted for season. Furthermore, preliminary analysis of skin pigmentation measures has revealed that risk of prostate cancer was associated with measures of skin pigmentation (P=0.02), and this effect is modified by sun exposure. We are examining polymorphisms in genes involved in the physiological response to UVR, these include VDR and genes involved in determining skin type, MC1R and tyrosinase. Preliminary analysis of genotyping data is currently underway and UVR exposure and skin type together with MC1R and VDR genotyping data will be presented.

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The Trp64Arg polymorphism of the beta 3-adrenergic receptor gene is associated with weight loss by health promotion intervention in obese Japanese men. *M. Yamakita, S. Tang, D. Ando, Z. Yamagata.* Health Sci, Univ. Yamanashi, Yamanashi, Japan.

It has been reported that the Trp64Arg gene polymorphism of the beta 3-adrenergic receptor gene was associated with impairment of its lipolytic function, a lower resting metabolic rate, and tendency to gain weight in obese individuals. Several studies have suggested that obese subjects with this polymorphism have difficulty in losing weight, but other studies have not supported this. Thus, it is unclear whether this gene polymorphism is associated with difficulties of weight loss. In this study, we investigated the relationship between the Trp64Arg gene polymorphism and weight loss by health promotion intervention in obese Japanese men. The subjects were 615 Japanese men aged 20-56 years who received general health check-ups in a company. In the company, health promotion intervention such as dietary intervention programs and exercise promotion campaigns conducted from 1999. The dietary intervention will affect health-related dietary knowledge, provide practical skills with which to change the diet, and facilitate or maintain behavioral changes through environmental programs. The plan for a cafeteria was to provide leaflets, to leave advertising menus at point of purchase and on tables in the cafeteria, and to put up posters on the walls. Genomic DNA was extracted from peripheral blood leukocytes. Trp64Arg genotype was determined by denaturing high-performance liquid chromatography (DHPLC) and PCR-RFLP. The subjects with BMI>25 kg/m^2 classified as obese. At the baseline of BMI, body weight, systolic blood pressure, diastolic blood pressure, there was no significant difference in participants with/without Trp64Arg polymorphism of the beta 3 adrenergic receptor gene. In analysis of covariance, significant difference was found in BMI change in Trp64Trp group (-0.30 kg/m^2), but not in Trp64Arg group (+0.54 kg/m^2) after adjusted for age, baseline BMI. We concluded that the Trp64Arg polymorphism of the beta 3-adrenergic receptor gene is difficult to weight loss by health promotion intervention in obese Japanese men.

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Heritability and Pleiotropy of Muscle and Bone Related Traits in Afro-Caribbean Families. *X. Wang[1], C.M. Kammerer[1], V.W. Wheeler[3], A.L. Patrick[3], C.H. Bunker[2], J.A. Cauley[2], J.M. Zmuda[2].* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Epidemiology, Univ Pittsburgh, Pittsburgh, PA; 3) The Tobago Health Studies Office, Scarborough, Tobago, West Indies.

The loss of bone and muscle with aging is a major contributor to increased morbidity and mortality. Bone and muscle mass are heritable polygenic traits, and muscle forces on the skeleton are among the strongest signals to stimulate bone growth. Despite the close relationship between muscle and bone, studies examining the potential pleiotropic effects of genes on muscle and bone related traits have yielded conflicting results. To address this issue further, we conducted quantitative genetic analyses to partition the phenotypic variance of muscle mass and bone size into their additive genetic and environmental components using maximum likelihood variance decomposition methods. State-of-the-art peripheral quantitative computed tomography (pQCT) was used to measure muscle cross-sectional area (mCSA) and periosteal circumference (PC) of the proximal tibia in 404 individuals aged 18 and older (mean, 43 years) from 8 large, multigenerational Afro-Caribbean families with a mean family size of >50 individuals (range from 21 to 112). Measured covariates (age, gender, height, weight) explained 50% of the variance in both mCSA and PC. The residual heritabilities of mCSA and PC were also moderately high: 0.40 0.10 and 0.67 0.10, respectively ($P<0.01$ for both traits). Besides, there was a strong positive genetic correlation between mCSA and PC (rhoG = 0.75 0.12; $P<0.001$) that was also significantly different from 1.0. Our results suggest that as much as 56% of the joint phenotypic variation in muscle mass and bone size may be influenced by a common set of genes. Bivariate genetic analysis of these pQCT related phenotypes should lead to the identification of genes for bone growth and osteoporotic risk.

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Macrophage scavenger receptor 1 (MSR1) haplotypes and prostate cancer (PC) risk in African Americans and Caucasians. *H. Rennert, C.M. Zeigler-Johnson, S.B. Malkowicz, T.R. Rebbeck.* University of Pennsylvania, Philadelphia, PA.

MSR1 plays a major role in inflammation, and was proposed as a candidate gene for hereditary PC. Germline mutations and sequence variants in MSR1 have been reported to be associated with PC risk and disease severity. Though several SNP-disease association studies have been reported for MSR1, very little is known about the association of MSR1 haplotypes with PC risk. We characterized MSR1 haplotypes using 5 SNPs (-14,742A>G, 520G>T, 876C>T, IVS5-59C>A, IVS7delinsTTA) with frequencies >1%. We examined the relationship between these haplotypes and PC risk in a case-control sample of 769 African American (AA) and European American (EA) men, using the EM algorithm as implemented by hapipf in STATA 8. Over ten haplotypes were observed in each race, with frequencies that varied significantly by race:

Haplotype	EA Cont (N=243)	EA Cases (N=382)	AA Cont (N=88)	AA Cases (N=55)
A.G.C.C.delTTA	0.84	0.85	0.59	0.60
A.G.C.C.insTTA	0.04	0.04	0.14	0.15
G.G.C.C.delTTA	0.05	0.05	0.15	0.18

Five haplotypes occurring at a frequency >1% accounted for >98% of all haplotypes in EA and AA. No overall difference in the distribution of MSR1 haplotypes by case status, grade, or by stage was observed in either race. This is in agreement with the negative association between PC risk and these sequence variants observed in the single SNP-based analyses. In contrast, in single-SNP based analyses IVS7delTTA had an elevated risk for low-stage and low-grade disease, while 876C>T was associated with high-grade PC. Because the SNP-based effects were by disease severity, future haplotype studies should employ larger sample-size, considering analysis by PC severity.

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Gene-environment Interaction between Asthma, Socioeconomic Status and Ancestry in Latino and African American Populations. *S. Choudhry[1], M. Naqvi[1], J. Kho[1], N. Ung[1], H.J. Tsai[1], S. Nazario[2], M. LeNoir[3], H.G. Watson[4], S. Thyne[1], K. Meade[5], W. Rodriguez-Cintron[2], N. Risch[1], E. Ziv[1], E.G. Burchard[1].* 1) University of California, San Francisco; 2) San Juan VAMC, Puerto Rico; 3) Bay Area Pediatrics, Oakland; 4) The James A. Watson Wellness Center, Oakland; 5) Children's Hospital and Research Institute, Oakland.

Asthma is a common respiratory disease caused by an interaction of genetic and environmental factors. Among U.S. children, asthma has been shown to be most prevalent in low income and minority groups. In the U.S., Puerto Ricans have the highest (11.2%), African Americans have an intermediate (5.9%), and Whites and Mexican Americans have the lowest asthma prevalence rates, 3.3% and 2.7%, respectively. Puerto Rican, Mexican American and African American are admixed populations and share varying proportions of African, European and Native American ancestries. We hypothesized that socioeconomic status (SES) may mediate the relationship between asthma and ancestry in admixed populations. We analyzed 360 Puerto Rican, 362 Mexican American and 352 African American asthma cases and controls. Individual admixture for each subject was estimated using up to 44 ancestry informative markers and the program STRUCTURE. SES was assigned using the FFIEC Geocoding/Mapping System based on median family income. Multivariate regressions were performed to test for an association between asthma, SES and ancestry and to look for an interaction between ancestry and SES. We found an interaction between SES and ancestry that was significantly associated with asthma in Puerto Ricans ($p= 0.003$), a trend for Mexican Americans ($p= 0.16$) and nothing significant for African Americans ($p= 0.85$). Most interestingly among Puerto Ricans, there was a significant trend towards higher European and lower African ancestry in cases with moderate and middle SES categories and lower European and higher African ancestry in cases with upper SES category ($p= 0.0005$ and 0.003). These results suggest that there may be a gene-environment interaction between asthma, SES and ancestry that may help explain part of the racial/ethnic variation in asthma prevalence.

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Admixture and Breast Cancer Risk Among Latinas in the San Francisco Bay Area. *E. Ziv[1], E. John[2], S. Choudhry[1], J. Kho[1], W. Lorizio[1], S. Huntsman[1], H. Matallana[1], E. Gonzalez-Burchard[1].* 1) Center for Human Genetics & Dept Medicine, Univ California, San Francisco, San Francisco, CA; 2) Northern California Cancer Center, Union City, CA.

Breast cancer rates vary substantially among different ethnic groups in the U.S. Latinas are known to be an admixed population with European, Native American and African ancestry. We examined genetic ancestry among Latina breast cancer cases and controls to determine if individual genetic ancestry is associated with risk. The study included 244 Latina women diagnosed with breast cancer between 1999 and 2001 and reported to the Northern California Cancer Center and 333 Latina controls, age-matched and obtained from a population based sample in the same geographic region. 44 ancestry informative markers were used to estimate ancestry among cases and controls. We used a maximum likelihood approach to combine information across markers to estimate individual ancestry. To compare distribution of ancestry among cases and controls were performed using a non-parametric rank sum test. In the combined sample we estimated European ancestry as 52.8%, Native American ancestry as 41.1% and African ancestry as 5.6%. There was a non-significant trend towards higher European ancestry among cases (53.7%) compared with controls (52.9%). However, among pre-menopausal women there was significantly higher Native American ancestry among cases (45.3%) compared with controls (39.3%) (p=0.039). Among post-menopausal women there was a trend towards higher European ancestry among cases (55%) compared with controls (51.7%) (p=0.16). Adjustment for known reproductive and lifestyle factors attenuated the difference in post-menopausal women but did not attenuate the difference in ancestry among pre-menopausal women. Higher Native American ancestry and lower European ancestry is associated with breast cancer among younger, pre-menopausal Latina women. These results may be due to unmeasured environmental risk factors or to difference in genetic risk factors that increase a particular subtype of breast cancer among younger Latinas with higher Native American ancestry.

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An empirical Bayesian procedure to assess population substructure using single nucleotide polymorphisms. *Q. Zhang, J. Luo, R. Chakraborty, L. Jin, R. Deka.* Center for Genome Information, Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

To assess the population substructure, hierarchical Bayesian approaches have been widely applied to estimate F_{ST}. However, subjective priors are often used in these approaches. To avoid dependence on priors, we have implemented an empirical Bayesian method to estimate F_{ST}. A Monte Carlo EM algorithm is adopted to estimate the hyperparameters. In this approach, three levels of hierarchy are implemented. At the first level, the data denoted by x_{ik}, the number of chosen alleles at i^{th} locus in k^{th} population, is distributed as a binomial distribution with the allele frequency as the parameter. The second level specifies beta distribution for allele frequencies with F_{ST} and ancestral allele frequency as parameters. The ancestral allele frequency is drawn from a distribution consistent with the expected allele frequency spectrum of neutral alleles under mutation-drift equilibrium (infinite sites model). The third level denotes beta distribution with hyperparameters (,) for F_{ST}. The posterior distribution for F_{ST} can be generated through hybrid form of Gibbs sampling and Metropolis Hasting (MH) algorithm while hyperparameters are updated by Monte Carlo EM algorithm. To save computing time, an importance sampling approximation is adopted, so instead to rerun the Gibbs and MH sampler at each update of the EM sequence, the hyperparameters (,) can be updated through reusing the first generated sampler. Simulation studies with different numbers of unlinked single nucleotide polymorphism markers and different numbers of populations are ongoing now to check the deviation of the estimated F_{ST} from the true F_{ST} and compare with general Bayesian methods. Supported by NIH grant ES06096.

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Natural history of HFE-related hemochromatosis in the Atherosclerosis Risk in Communities (ARIC) Study. *J.S. Pankow[1], E. Boerwinkle[2], P.C. Adams[3], E. Guallar[4], J. Rogowski[1], J.H. Eckfeldt[1].* 1) Univ Minnesota, Minneapolis, MN; 2) Univ Texas Health Science Center, Houston, TX; 3) London Health Sciences Centre, London, Ontario, Canada; 4) Johns Hopkins Medical Institutions, Baltimore, MD.

The C282Y mutation of the HFE gene is a frequent cause of hemochromatosis, a disorder characterized by excess iron deposition in multiple tissues and organs that may result in clinical disease. We examined morbidity and mortality among C282Y homozygotes (C282Y/C282Y) in the ARIC Study, a population-based cohort study conducted in four U.S. communities that featured four examinations (visits 1-4) approximately 3 years apart. Subjects were 45-64 years old at visit 1 (1987-89) and were followed through 2002 for death, incident coronary heart disease (CHD), and stroke, through 1996-98 for incident diabetes, and through 2000 for incident cancer. Cox proportional hazards regression was used to model time to event for each outcome. The prevalence of homozygosity for the C282Y mutation was 0.41%; (45/10,999) in Caucasians, similar to other North American population-based studies. By contrast, the prevalence in African Americans (0.03%; 1/3,926) was too low to permit further analysis. No C282Y homozygotes were hospitalized with a discharge diagnosis of liver disease during the follow-up period. Rates of all-cause mortality, and incident CHD, stroke, and diabetes among Caucasians were similar in C282Y homozygotes and wild-type subjects. Although differences were not statistically significant, there was a modest excess of incident cancer among C282Y homozygotes (HR: 1.79; 95%CI: 0.93-3.44), particularly for breast cancer in women (HR: 3.05; 95%CI: 0.99-9.52) and prostate cancer for men (HR: 3.10; 95%CI: 0.99-9.68). At visit 1, LDL cholesterol levels were 15 mg/dl lower in C282Y homozygotes compared to wild-type subjects (p=0.009), a difference that remained statistically significant at later exams (11, 13, and 16 mg/dl at visits 2, 3, and 4, respectively). These population-based data suggest that the penetrance of any clinical disease in C282Y homozygotes is relatively low. Further investigation of possible HFE-related differences in cancer incidence and LDL cholesterol levels appears warranted.

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Optimum two-stage designs in case-control genetic association studies using false discovery rate. *A. Kuchiba[1], N.Y. Tanaka[2], Y. Ohashi[1].* 1) Dept Biostatistics, Univ Tokyo, Tokyo, Japan; 2) Dept Clinical Bioinformatics, Univ Tokyo, Japan.

Genetic association studies using case-control designs are often carried out to identify disease-susceptibility loci. In such studies, a large number of genetic markers can be used, and therefore result in increase of genotyping cost. Several types of two-stage designs are proposed and used from the point of cost-effectiveness. We propose the utilization of false discovery rate (FDR) with multiple-testing correction in two-stage designs, and show optimal sample sizes and criteria for selecting markers associated with the disease in each stage that minimize the cost of genotyping. The sample sizes and selection criteria is defined as a function of a prior probability that marker-disease association is true. In addition, the efficiency of the following confirmatory study can be considered by the definition of FDR. We compare the expected power and the expected cost of two-stage designs with those of one-stage designs under the assumptions that the genetic markers are independent and total sample size is fixed. The results show that the proposed two-stage procedure usually reduces the cost of genotyping by 40-60% with similar power of the one-stage designs. Also, we examine the effect of the correlation between markers if those exist, and the misspecification of a prior probability on the efficiency by using computer simulations.

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Novel screening method for whole genome candidate gene association studies. *N. Tanaka[1], T. Miki[2], T. Tokunaga[3].*
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With the development of high-resolution mapping of microsatellites and single nucleotide polymorphisms (SNPs) in the human genome, enormous amounts of gene polymorphism data with various diseases have become available. Then a large number of candidate genes can be subjected to whole genome candidate gene association studies with various diseases. Based on such extensive association analyses, the candidate regions are selected for further analysis. Many statistically methods, which have been proposed for association screening, are mostly based on the methods of statistical hypothesis testing so that the candidate regions are often selected based on the adjusted significance level. However, the statistical power is depended on the marker allele frequencies, which might be varying among genetic region, so infrequent and modest risk alleles are easy to be failed to detect. Furthermore, not only p-values but also odds ratios might be important measures for selecting the candidate regions. We propose here novel screening method for case-control association studies to select candidate genes based on smoothed odds ratios, which consider variance-covariance structures of the odds ratios estimated in each genetic region. Simulation studies were performed to compare the present method with other standard methods, having data from an extensive multi-institutional case-control association study on hypertension in Japanese. The present method provided better performance than the other methods in the situations that susceptibility-associated alleles are infrequent and exhibit only moderate risks to the disease.

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The Effect of Clinical and Brain White Matter Before and After Treatment in Patients with Tetrahydrobiopterin Deficiency. *Z. Zhang, W. Yu, Z. Zhou, X. Zhang.* Department of Pediatric, China-Japan Friendship Hospital, Beijing, China.

Objective To present MRI evidence on the effect of treatment by comparing the brain abnormalities of the white matter and myelination before and after treatment for one year in patients with BH4 deficiency using 0.5T MRI examination. Method Eleven patients with BH4 deficiency aged 17 weeks to 4 years were observed. Among the 11 cases , 9 were males and 3 were females .All cases were diagnosed as BH4 deficiency by analysis of urinary pterins, BH4 loading test and determination of dihydropteridine reductase in RBC. The patients undertook MRI examination before treatment with BH4, L-Dopa and 5-HTP, 8 cases were revaluated with the Gesell developmental scale. Results The result showed that the DQ of 8 cases have been developed after treatment. At the beginning, Eleven cases (100%) presented delayed myelination in frontal lobe, 8cases (72.7%) having delayed myelination in occipital lobe , 4 cases (36.4%) in temporal lobe and 3 cases(27.3%) in parietallobe. Delayed myelination of corpus callosum can be found in 6 cases(54.5%). There were abnormal diffuse high signal in white matter shown by T2W1 in all cases. Of the 9 patients reevaluated after one year treatment, delayed myelination were still present in 5 cases(45.5%) in frontal lobe, in 5 cases (45.5%) in occipital lobe, in 2 cases (18.2%) in temporal lobe, in 2 cases(18.2%) in parietal lobe. in 3 cases(27.3%) in corpus callosum. Abnormal diffuse high signal in white matter shown by T2W1 in 5 cases(45.5%). Conclusion All patients(100%) with BH4 deficiency present brain abnormalities in white matter, which is higher than the occurrence of patients with PKU. A possible explanation is that the impairment of decreased synthesis of neurotransmitters precipitate the brain abnormalities in white matter. Great improvement was seen in the white matter after one year treatment, which is consistent with the improvement in clinical manifestations.

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Development of a therapeutic bone-targeting system for mucopolysaccharidoses. *S. Tomatsu[1], M. Gutierrez[1], T. Nishioka[1], J. Grubb[2], W. Sly[2].* 1) Dept Pediatrics, Ped Res Inst, St Louis Univ, St Louis, MO; 2) Dept Biochem, St Louis Univ, St Louis, MO.

Current approaches for the treatment of mucopolysaccharidoses (MPS) have little impact correcting the bone pathology. Deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS) and -glucuronidase (GUS) responsible for MPS IVA and VII leads to the systemic skeletal dysplasia. Modifying the drug to be delivered to the bone more specifically is noteworthy to enhance the drug effectiveness. The aims of this study were to compare efficiency of delivery of GALNS and GUS enzymes to the bone between untagged and tagged enzymes(the small acidic peptide-six glutamines was tagged to N-terminus of mature protein), and to assess pathological improvements by using enzyme replacement on established MPS IVA and VII mouse models immunotolerant to human enzymes. Methods: 1. Purified GALNS and GUS derived from CHO cell lines were administered intravenously to respective mouse model at a single dose of 250 units/g of body weight. Treated mice were examined by assaying the enzyme activity at 0, 2, 5, 10, 20, 30, 60, 120, and 240 min to assess clearance of the enzyme in the blood stream. The mice were sacrificed 2, 24, 72, 168 hr after infusion of the enzyme for studies of the tissue distribution pattern of the enzymes. 2. To see the effectiveness of clearance of storage and reversal of phenotype, the 250 units/g dose was given through tail veins weekly for 12 weeks on mouse models. Mice were sacrificed seven days after the last infusion. Results: 1. The tagged enzymes had 5-10 times more prolonged clearance of half life time in blood and 10% of GUS enzyme activity retained in blood even after 6hr of injection. Tagged enzymes retained longer in bone and bone marrow, keeping 3 times higher enzyme activity in bone in 24hr compared to the untagged enzyme. 2. The pathological findings in mice treated with tagged enzymes showed the more clearance of the storage materials in bone and interestingly, cornea as well, compared to those with untagged enzymes. Conclusion: The results of pre-clinical trials on the MPS IVA and VII mouse models indicate the effectiveness of this bone-targeting strategy.

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Animal Study of Direct Central Nervous System Administration of Copper Histidine: Prelude to Clinical Trial in Menkes Disease. *K. Lem[1], L.R. Brinster[2], O. Tjurmina[3], S.G. Kaler[1].* 1) Unit on Pediatric Genetics, NICHD/NIH; 2) Division of Veterinary Resources, NIH, Bethesda, MD; 3) Center for Scientific Review, NIH, Bethesda, MD.

BACKGROUND: Classical Menkes disease (MD) is a fatal neurogenetic disorder of infancy caused by mutations in a copper transporter gene (ATP7A) associated with brain copper (Cu) deficiency. Subcutaneous Cu injections (500 mcg q.d.) from early infancy can prevent neurodegeneration in some MD infants. Success of this treatment is mutation-dependent; patients with null defects do not respond favorably. Post-mortem tissue analyses in such treatment failures indicated that copper failed to traverse the blood-brain barrier. OBJECTIVE: To assess the safety of copper administration to the CNS as a therapeutic approach for MD infants with null ATP7A mutations, we performed toxicologic studies of intraventricular copper histidine (CuHis) in healthy adult rats. DESIGN/METHODS: We used stereotaxic brain surgery to implant cannulae into the R lateral ventricles of 36 adult male rats, and infused 20 microliters of CuHis or normal saline over 10 minutes. To determine maximum tolerated dose (MTD), 0.5,1.0, 2.5, 5.0, 7.5, 10 or 100 mcg of CuHis was administered; brains were harvested and examined after 6 hrs. To assess longer term effects, 10 rats received 5 mcg CuHis and were euthanized after 14 days. RESULTS: Surgeries and infusions were well tolerated. On clinical grounds, the MTD for intraventricular CuHis was 5 mcg. Compared to saline-treated controls, rats receiving the MTD showed mild periventricular inflammatory changes after 6 hrs. After 14 days, ventricular dilatation, neuronal cell loss, spongiform changes, and dense periventricular inflammatory cell infiltrates were evident. In contrast, with a dose of 0.5 mcg CuHis, histologic findings were much less severe and, in some instances, indistinguishable from saline-infused controls. CONCLUSIONS: We established a MTD (5 mcg) of intraventricular CuHis in adult rats. Histopathological analyses suggested that a 0.5 mcg dose is safer and better tolerated. These results provide guidance concerning intrathecal copper therapy for classical MD.

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Towards therapy of Smith-Lemli-Opitz syndrome with combination high cholesterol diet and simvastatin. *A. Pappu[1], W. Connor[1], L. Merkens[2], J. Penfield[2], J. Jordon[1], R. Steiner[2, 3].* 1) Dept of Med, OHSU, Portland, OR; 2) Dept of Ped,OHSU, Portland, OR; 3) Dept of Mol & Med Gen, OHSU, Portland, OR.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive condition, with a deficiency of 7-dehydrocholesterol reductase, which converts 7-dehydrocholesterol (7DHC) to cholesterol (chol). The resultant low chol and high 7DHC levels in plasma and tissues may be responsible for the phenotypes. One treatment objective of SLOS is to raise plasma chol levels by increasing dietary chol intake and simultaneously lower 7DHC levels. A frequently used measure of disease severity and response to therapy in SLOS is the 7DHC/chol ratio, a low ratio being desirable. Statins inhibit HMG CoA reductase (HMG CoAR), a key regulatory enzyme in the chol biosynthetic pathway, and can be monitored by measuring the enzyme product, mevalonate, in urine (UMVA). We selected the lipophyllic simvastatin (simv) as the statin of choice to study in SLOS because of its potency in inhibiting HMG CoAR, its ability to pass the blood brain barrier. Five subjects with SLOS were studied on three different dietary regimens: a very low chol diet, a high chol diet, and a combination high chol diet with simv. The effects of the interventions were determined by measuring plasma sterols and UMVA and by monitoring for adverse effects. In these subjects, changing the diet from low to high chol resulted in a 24% increase in plasma chol, 12% decrease in plasma 7DHC, and 51% decrease in UMVA. A combination of high chol diet and simv maintained plasma chol levels while reducing 7DHC by 28% and UMVA by 67%($p<0.02$). Simv did not lower chol in SLOS as it does in FH. Simv reduced the ratio of plasma 7DHC/chol by 40% vs 31% reduction with high chol diet alone ($p<0.01$). There was a significant correlation between %reduction in plasma 7DHC and UMVA ($r=0.7$, $p=0.02$). We conclude that feedback inhibition of HMG CoAR by dietary chol results in reduced substrate flux through the chol biosynthetic pathway leading to reduced 7DHC, and that these effects are augmented by simv. We further conclude that a combination of high chol diet and simv has additive beneficial effects to a high chol diet and shows preliminary evidence of safety.

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Locus Specific Databases: from mutations to gene therapy by exon-skipping. *C. Beroud, D. Hamroun, S. Tuffery-Giraud, A. Disset, G. Collod-Béroud, M. Claustres.* Dept of Molecular Genetics, CHU de Montpellier, Montpellier, France.

Thousands of mutations are identified in diagnostic and research laboratories yearly. To collect and analyze these data we developed a generic software called Universal Mutation Databases (UMD) to create locus-specific databases (http://www.umd.be). This freely available tool allows the creation of LSDBs for virtually any gene and includes a large set of analysis tools. Thus, we have created specific routines to help researchers to design new therapeutic strategies such as exon skipping. The archetype for exon skipping is the DMD gene associated with Duchenne (DMD) and Becker dystrophies (BMD). Most of the DMD mutations consist in large genomic deletions. The out-of-frame deletions lead to premature stop codons that result in the severe phenotype (DMD) while deletions that produce in frame mRNAs leading to shorter proteins are responsible for a milder myopathy (BMD). In patients as well as in animal models, rare dystrophin-positive fibers have been reported. It has been suggested that restoring the reading-frame by exon skipping is the most likely cause of this natural phenomenon. Therefore many groups are designing strategies for gene repair/modulation based on the use of compounds interfering with splicing, thus inducing exon skipping. Because the DMD gene contains 79 exons, exon skipping can produce more than 3.000 potential transcripts. We developed various tools to select the best exon(s) skipping(s) to rescue the largest number of DMD patients. If the skipping of exons can be carried out by various approaches, the choice of the target sequences is of primary importance. Because algorithms searching for ESE, ESS and branch points are based on consensus sequences, it results a strong proportion of false positive signals. To optimize these algorithms, we developed collaborations with various teams able to identify functional sequences and validate software predictions. The combination of fully documented molecular and clinical data from DMD and BMD patients, the in vitro assays and bioinformatics approaches should thus make it possible to identify the best target sequences in order to consider clinical trials of patients.

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Intracranial Pressure and Late Ophthalmalogic and Neurologic Outcome in Hurler Syndrome after Bone Marrow Transplantation. *L. Charnas[1], K.G. Belani[2], D. Beebe[2], C.G. Summers[3], S.J. Haines[4], C.B. Whitley[1].* 1) Deparments of Pediatrics,; 2) Anesthesiology; 3) Ophthalmalogy; 4) Neurosurgery, Univ of Minnesota, Minneapolis, MN.

Background: Elevated ICP and hydrocephalus occur in MPS I but the time course of resolution and long term effects on cognition and vision after bone marrow transplantation are poorly understood. **Methods:** Serial lumbar punctures (LP) were obtained in 19 children with Hurler syndrome undergoing bone marrow transplantation. LPs under general anesthesia with end tidal CO_2 between 30 -35 mm Hg were performed prior to transplant and at days 28, 100, and 200 in all patients during measurements of CSF glycosaminoglycans and then yearly until ICP was normal. **Results:** No infectious or bleeding complications were associated with lumbar puncture in these patients. 8 of 19 patient had elevated CSF pressures prior to transplant (> 20 cm H_2O) while 5 of the 8 had significant elevation (> 30 cm H_2O). Three patients were treated with ventriculoperitoneal shunts for elevated ICP without clinical symptoms. Nine patients had ventriculomegaly by cranial CT scan, with six of the nine showing decreased perventricular attenuation suggesting periventricular edema. Papilledema was identified in 6 children. Four other children demonstrated optic atrophy. OFC and fontanel size correlated poorly with ICP. ICP > 30 cm H_2O was associated with papilledema and optic atrophy but did not predict visual outcome. ICP normalized in all children by 18 months post transplant. Cognitive outcome was not statistically associated with elevated ICP or its treatment. **Conclusions:** Serial lumbar puncture is safe in the acute transplant period. Mild ICP elevation (< 30 cm H_2O) resolves with transplantation without adverse outcome. Significantly elevated ICP (> 30 cm H_2O) can be associated with optic nerve findings. Additional factors other than increased ICP also contribute to adverse visual and cognitive outcome.

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Does the blood group phenotype affect the ex-vivo expansion or clonogenic capacity of umbilical cord hematopoietic stem cells? *J. Santolaya-Forgas[1], I. Galan[2], J. De Leon[2], R. Hume[1].* 1) Prof OB/GYN & Pediatrics, Wayne State University, Detroit, MI; 2) Amarillo Women's Health Research Institute, Texas Tech Health Science Center at Amarillo, TX.

Objective: Umbilical cord hematopoietic stem cells, UCHSC (CD34positive,Lin-), have been used for allogenic transplantation in inherited disease. The effect of the blood group phenotype on the proliferative and clonogenic capacity of hematopoietic stem cells is not known. Our aim was to evaluate the ABO antigenic system effect on umbilical cord derived stem cell ex-vivo response to growth factors.

Study Design: UCHSC were collected from 3 placenta with different ABO blood groups under a Texas Tech IRB#2041 protocol. CD34 cell isolation following standard methods. 125 thousand cells from each cord were first cultured for 7days in platforms containing a 3-D biocompatible matrix coated with full-length fibronectin, RPMI with fetal bovine serum and one of 4 different sets of hematopoietic growth factors (Stem cell factor, thrombopoietin, IL3, IL6 and FLT3ligand). Ten thousand viable cells from each blood group were then seeded on the platforms for 7 days in each condition. Dunn's Multiple Comparison Test was used.

Results: Freshly prepared cord blood CD34positive,Lin- cells were planted and cultured efficiently in all platforms. O positive cells had greater expansion in all four culture conditions (cells counts: O 425k, B 315k, A 320k; $p<0.05$). UCHSC self-renewal for O, A and C cells were comparable (percentage: 23, 21 and 19; $p>0.05$).

Conclusion: This study suggests that the antigenic load within the cell membrane may determine the proliferative capacity of hematopoietic stems cells in response to growth factors. Clinical studies to determine if UCHSC from universal blood donors (O, Rh-negative) lead to functional hematological reconstitution in recipient patients in shorter periods of time may have important prognostic value. Lineage of differentiation optimization may require specific culture conditions.

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A follow-up study: Can donepezil improve quality of life of Down syndrome patients? *T. Kondoh[1], M. Kojima[2], A. Tanaka[1], M. Nakashima[3], K. Aikawa[2], H. Sasaki[3], H. Moriuchi[1].* 1) Dept Pediatr, Nagasaki Univ Hospital, Nagasaki, Japan; 2) Faculty of Education, Nagasaki Univ, Nagasaki, Japan; 3) Dept Hosp Pharmacol, Nagasaki Univ Hosp, Nagasaki, Japan.

This is a follow-up report of the clinical trial of donepezil therapy to a total of 21 Down syndrome (DS) patients for 36 months. Our protocol of donepezil therapy for DS patients is described as below: (1) AST, ALT, LD, BUN, Cr, Amy, fT3, fT4, TSH and ECG are checked as pre-treatment examinations. (2) The loading dose of donepezil is usually 3 mg daily. (3) The aforementioned examinations and plasma donepezil concentrations are checked at 24 h after administrations of the last dose on the 28th treatment day. (4) Their general condition is checked by physical examinations and interviews from their parents at regular hospital visits. Their QOL is assessed by Tanaka-Binet test, Picture Vocabulary test, Social Maturity scale, and Adaptive Behavior scale. When efficacy of donepezil is considered suboptimal, necessity of dose-up is evaluated in consideration of adverse effects and plasma concentration. (5) When therapeutic dose is changed, plasma concentration is measured after one month. Our 36-month clinical trial yielded four implications. First, when DS patients have liver dysfunction, donepezil treatment should be prudently monitored. One DS patient with fatty liver suffered from nonalcoholic steatohepatitis although causal relation was unknown. Second, plasma donepezil concentrations tend to be higher in DS patients than in normal controls. Since such distinct pharmacokinetics may contribute to the likelihood and severity of adverse effect, careful monitoring and dose adjustment will be critical for the safe use. Third, subjective assessment by parents under the double-blinded condition indicated efficacy to improve the ability of daily activity in many DS patients. Furthermore, increase in scores of adaptive behavior scales or Binet intelligent scale was noted in some DS patients after donepezil therapy. Fourth, donepezil might also be effective in some DS patients with rapidly progressive deterioration. In conclusion, donepezil is promising for improvement of QOL of some DS patients.

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Therapeutic strategies based on mRNA splicing modification in human genetic disease. *S. Slaugenhaupt[1], M. Hims[1], E. Ibrahim[2], M. Leyne[1], J. Mull[1], S. Gill[1], L. Liu[1], R. Reed[2].* 1) Center for Human Genetic Research, Massachusetts General Hospital; 2) Department of Cell Biology, Harvard Medical School.

It has been estimated that approximately 15% of all mutations that lead to human genetic disease alter mRNA splicing. Further, the role of alternative splicing in creating genetic complexity is highlighted by the lower than expected estimate of the number of human genes following the completion of the human genome sequence. Our studies have focused on familial dysautonomia (FD), a hereditary sensory and autonomic neuropathy that is caused by a splice mutation in the *IKBKAP* gene. The mutation results in variable skipping of exon 20 in *IKBKAP* mRNA, which leads to a tissue-specific reduction of IKAP protein. The fact that FD patients retain the capacity to make both normal mRNA and protein offers an exciting, direct approach towards therapy. Using both *in vitro* and *in vivo* minigene splicing models we have shown that in its native state exon 20 is very weak, and that we can correct exon 20 skipping using a variety of sequence alterations.

Last year we reported that kinetin, a plant cytokinin, enhances exon 20 inclusion and dramatically increases the amount of wild-type *IKBKAP* mRNA and IKAP protein in FD cells. Hybridization of Affymetrix chips with RNA isolated from kinetin treated cells has identified two splicing factors with altered expression in response to kinetin. We are currently cloning and expressing these factors to determine if they directly alter *IKBKAP* splicing. Studies using *IKBKAP* minigene constructs show that the ability of kinetin to enhance splicing efficiency is not dependent on either the presence of the FD mutation or the wider regulation of *IKBKAP* transcription or translation. Recent evaluation of a panel of deletion constructs has allowed us to identify a specific sequence element in exon 20 that is required for kinetin activity. Precise characterization of this sequence element will permit *in silico* analysis which will lead to the identification of other human disease genes harboring splice mutations that might respond to kinetin.

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Pre-clinical validation of a multiplex real-time RT-PCR assay to quantify SMN transcripts in whole blood samples from SMA patients. *L.R. Simard[1], M-C. Bélanger[1], M. Wride[2], K.J. Swoboda[2].* 1) Dept Ped, Ctr de Recherche, Hopital Ste-Justine, Montreal, PQ, Canada; 2) University of Utah, Salt Lake City, Utah, USA.

SMN2, a duplicate copy of *SMN1*, is the only known modifier gene of Spinal Muscular Atrophy (SMA), a devastating childhood neurodegenerative disease; a premise supported by the inverse relationship between *SMN2* copy number and disease severity. Consequently, *SMN2* is an attractive therapeutic target and drug screens for compounds that alter SMN splicing or up-regulate *SMN* expression led to the proposed use of phenylbutyrate (PBA) and valproic acid (VPA) to treat SMA. As these drugs are expected to increase the amount of full-length SMN protein, we developed a multiplex real-time RT-PCR assay to quantify SMN transcripts in whole blood samples drawn from SMA patients. Published data indicated an increase in full-length SMN mRNA of about 2-fold; thus, we determined whether SMN expression was stable over time and established an experimental design to minimize variability between real-time measurements. Briefly, we exploited the PAXgene^{TM} Blood RNA system (QIAGEN) to obtain RNA samples from patients recruited by multiple centers across North America. Blood samples were sent to a centralized laboratory equipped to run real-time experiments; shipments occurring within a 24 hour period. Overall, we obtained good quality and sufficient amounts of total RNA. These RNA samples were stable for a >2 year time interval. The latter allows one to assay SMN transcripts from patient samples collected over a 9 to 12 month period in a single run, thus avoiding inter-experimental variability. Total mRNA was prepared and the amount of full-length SMN transcripts detected using a fluorescent probe spanning the 7-8 exon boundary. Efficiency of the reverse transcription and PCR reactions were monitored by simultaneously measuring phosphoglycerate kinase (PGK1; ABI) transcripts. In general, SMN expression varied minimally over a >12 month period indicating that this assay can be used to follow the effect of drug treatment on SMN expression in SMA clinical trials. Detailed findings will be presented so that uniform protocols can be adopted across different SMA drug trials. Funded by Families of SMA.

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Long term dietary cholesterol supplementation in Smith-Lemli-Opitz syndrome. *S. Sparks[1], H. Goodwin[2], C. Wassif[2], A. Gropman[3], E. Tierney[4], F. Porter[2].* 1) NHGRI/NIH, Bethesda, MD; 2) NICHD/NIH, Bethesda, MD; 3) Georgetown University, Washington, DC; 4) Kennedy Krieger Institute, Baltimore, MD.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis. The defective enzyme, 3-hydroxysterol 7-reductase encoded by *DHCR7*, is responsible for the final step of the conversion of 7-dehydrocholesterol to cholesterol. Children with SLOS have elevations of dehydrocholesterol and decreased serum and tissue cholesterol, resulting in the characteristic clinical and behavioral features of SLOS. Current therapy of SLOS patients consists of dietary cholesterol supplementation. We phenotypically characterized 50 patients with SLOS at the NIH Clinical Center and evaluated the long term efficacy of dietary cholesterol supplementation on the biochemical and clinical aspects of SLOS. All patients were placed on dietary cholesterol supplementation (150-200 mg/kg/d) using a suspension of crystalline cholesterol in Ora-Plus. Patients were evaluated every 6-12 months. Plasma cholesterol, 7-dehydrocholesterol and 8-dehydrocholesterol were determined using GC/MS. For group comparison, sterol levels were normalized to initial values at diagnosis. Patients were categorized as mild (n = 29), classical (n = 15) or severe (n = 6) based on physical manifestations. Genotypes and residual DHCR7 activity were determined for all patients. Decreased enzyme activity correlated with increased clinical severity. In mild SLOS patients, dietary cholesterol treatment for 60 months resulted in an initial increase in cholesterol which peaked at 24 months and subsequently fell. Dehydrocholesterol levels remained relatively constant. In classical SLOS patients treated with cholesterol for 60 months, the cholesterol level gradually increased to approximately 2.5 fold over baseline. Dehydrocholesterol levels remained unchanged. Dietary cholesterol supplementation over 24 months in severe SLOS patients also resulted in increased cholesterol levels to 2 fold above initial levels and dehydrocholesterol levels that were unchanged. Dietary cholesterol may have beneficial effects on children with SLOS, however it does not affect the dehydrocholesterol levels.

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Progressive Mesenteric and Mediastinal Lymphadenopathy Despite Enzyme Therapy in a Patient with Gaucher Disease. *T. Burrow, M. Cohen, A. Choudhary, G. Deutsch, R. Bokulic, G. Grabowski.* Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Enzyme therapy (ET) in Gaucher disease (GD) has led to remarkable improvements in the hepatic, splenic, hematologic, and bony involvement in affected patients. However, the CNS, lung, and in certain cases, the bone, have demonstrated a deficient response to ET and may be relatively inaccessible compartments. The lymph node system also appears, in selected patients, to be an inaccessible compartment. A patient with subacute neuronopathic (type 3) GD received ET (60 U/kg) every two weeks from 1 year of age. By 5 years of age, his massive hepatosplenomegaly and hematologic abnormalities had resolved. Beginning at 3 years of age, he experienced intermittent bouts of diarrhea and abdominal bloating as well as slowly progressive bronchospastic disease. Chest x-ray and MRI showed progressive interstitial infiltration of the lungs and mediastinal lymph node enlargement. By 4 years of age, extensive mesenteric and retroperitoneal lymphadenopathy was present, which progressed to massive replacement of the mesentery and lymph nodes with calcified masses within one year. This was coincident with the development of hypoalbuminemia without hepatic or renal dysfunction, indicative of a protein losing enteropathy. Exploratory laparotomy to rule out malignancy showed replacement of the mesentery with massive lymph nodes. Histopathology of these mesenteric masses showed lymph nodes replaced by sheets of macrophages, some resembling Gaucher cells with PAS positive proteinaceous material and others containing cholesterol crystals and lipid droplets. This suggested to us that the lymphadenopathy was due to the relative inaccessibility of the lymph nodes to ET. The hypoalbuminemia was stabilized with an MCT containing diet. The use of increased ET (60 U/kg weekly) and substrate depletion approaches are being evaluated. Such unusual cases highlight the need for comprehensive therapies in affected patients and the presence of poorly accessible compartments in patients with GD and other lysosomal diseases receiving ET or other therapeutic modalities.

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ER retention and aggregation induced apoptosis associated with neuropathy causing MPZ truncating mutants are abrogated by curcumin treatment. *M. Khajavi[1], W. Wiszniewski[1], T. Ohyama[1], K. Inoue[2], G. Jackson Snipes[3], J.R. Lupski[1, 4, 5].* 1) Deptartment of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan; 3) Deptartment of Pathology, Baylor College of Medicine, Houston, TX; 4) Texas Childrens Hospital, Houston, Texas; 5) Deptartment of Pediatrics, Baylor College of Medicine, Houston, TX.

Mutations in *MPZ*, encoding myelin protein zero the major protein constituent of peripheral myelin, can cause the adult onset inherited neuropathy Charcot-Marie-Tooth (CMT) disease as well as a more severe childhood onset Dejerine-Sottas Neuropathy (DSN) and congenital hypomyelinating neuropathy (CHN). Most *MPZ* truncating mutations associated with severe forms of peripheral neuropathy result in premature termination codons (PTCs) within the terminal or penultimate exons that are not subject to nonsense mediated decay (NMD) and are stably translated into mutant proteins with a potential dominant-negative activity. However, some truncating mutations at the 3 end of MPZ escape the NMD pathway and yet cause a mild peripheral neuropathy phenotype. We examined the functional properties of MPZ truncating proteins that escaped NMD and found that frameshift mutations associated with severe disease cause intracellular accumulation of mutant proteins, primarily within the endoplasmic reticulum (ER), that induces apoptosis. Curcumin, a chemical compound derived from the curry spice tumeric, releases the ER-retained MPZ mutants to the cytoplasm and is accompanied by a lower number of apoptotic cells. Our findings suggest that curcumin treatment is sufficient to relieve the toxic effect of mutant aggregation induced apoptosis and may potentially have a therapeutic role in selected forms of inherited peripheral neuropathies.

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Anderson-Fabry disease - changes in typical facial features in response to enzyme replacement therapy. *A.G. Milligan, M. Blincoe, R. Bruce, S. Goodwin, A. Holmes, D. Hughes, M. Reed, L. Richfield, A. Mehta.* LSDU, The Royal Free Hospital, London, United Kingdom.

INTRODUCTION: Deficiency of alpha-galactosidase-A results in deposition and progressive accumulation of globotriaosylceramide and related glycosphingolipids in many tissues with a predilection for vascular endothelial and smooth muscle cells resulting in the clinical manifestations of Anderson-Fabry disease. Many adult hemizygous males develop typical coarse facial features similar to those observed in patients with acromegaly. In acromegaly high levels of insulin-like growth factor-1 (IGF-1) are found and this cytokine leads to growth and differentiation of many different cell lines including keratinocytes and fibroblasts. METHODS: In order to document changes in facial appearances in response to enzyme replacement therapy facial photographs were taken by a medical photographer prior to commencing treatment with agalsidase alpha and serially following 6- 12 months of therapy. Written consent was obtained from all patients. RESULTS: The serial photographs show a noticeable change in the facial features. There is clear increase in definition of the nasal bridge and reduction in peri-orbital swelling and supra-orbital soft tissue. The patient in figure 2 had gained considerable weight between follow up visits and the changes are less obvious. Patient 3 shows changes over 24 months of agalsidase alpha. CONCLUSIONS: Clearance of storage material (Gb3) from other tissues and organ systems with agalsidase alpha is well documented with measurable improvement in clinical parameters such as left ventricular mass. Serial medical photography of facial features in those patients with the typical Fabry facies at baseline and at 6 to 12 monthly intervals of treatment demonstrate a gradual and subtle change in facial features. Whilst serial single dimensional color photography is a useful tool in documenting these changes the interpretation is subjective, more refined techniques using 3 dimensional images will provide quantitative evidence of response to therapy.

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Intrathecal Enzyme Replacement Therapy in a Patient with Mucopolysaccharidosis and Symptomatic Spinal Cord Compression. *R. Giugliani[1], M.V. Munoz R.[1], P. Dickson[2], T. Vieira[1], R. Costa[1], L. Vedolin[3], S. Canani[1], L. Jardim[1], E. Kakkis[4].* 1) Medical Genetics Service, Hosp Clin P Alegre, Porto Alegre, RS, Brazil; 2) Division of Medical Genetics, LA Biomed. Inst. at Harbor-UCLA, Torrance, CA, USA; 3) Imaging Service, Mae de Deus Center, Porto Alegre, RS, Brazil; 4) Biomarin Pharmaceutical Inc., Novato, CA, USA.

Introduction: In MPS I, deficiency of -L-iduronidase and subsequent glycosaminoglycan (GAG) storage can cause spinal cord compression in the cervical meninges. Surgical treatment carries a high risk of morbidity and mortality. As intravenous enzyme replacement therapy (ERT) is not likely to cross the blood-brain barrier, we investigated intrathecal recombinant human -L-iduronidase (IT rhIDU) in an MPS I patient with spinal cord compression. To our knowledge, IT therapy in humans has not been attempted previously for this disorder. Purpose: To evaluate the safety and efficacy of IT rhIDU for spinal cord compression caused by cervical meningeal storage in a MPS I - Scheie patient. Methods: We assessed a MPS I - Scheie patient with spinal cord compression at baseline with clinical, biochemical evaluations, 4-extremity somatosensory evoked potentials (SSEP), 12 minute walk test (12MWT) and conventional MRI and diffusion tensor imaging (DTI) studies of the CNS. He was monitored for changes in these parameters during 4 IT infusions of rhIDU administered monthly via lumbar puncture (LP). Results: No adverse events were observed. After 3 infusions he reports improvements in cord compression symptoms, including decreased numbness and tingling, increased stability when rising from a chair, and ability to reduce the frequency of his pain medication. CSF opening pressure at baseline was normal (130 mm H2O), decreasing to 90 mm by the third LP. There were no clinically significant changes in serum chemistries or CSF protein, glucose, or cell count. 12MWT presents a modest (~7%) improvement so far, and CNS imaging studies reveal no progression of hydrocephalus or white matter lesions. Further evaluation is ongoing, and available results will be presented. Conclusion: IT rhIDU appears to improve spinal cord compression in this MPS I patient.

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Monthly, quarterly, and low-dose intrathecal iduronidase for canine MPS I. *S. Le[1], P. Dickson[1], C. Vogler[2], B. Levy[2], M. McEntee[3], C. Guerra[1], M. Peinovich[1], C. Jagabat[1], H. Manuel[1], E. Kakkis[4].* 1) Med. Genetics, LA Biomed at Harbor-UCLA, Torrance, CA; 2) Dept. of Pathol., St. Louis Univ., St. Louis, MO; 3) Dept. of Pathol., Coll. of Vet. Med., Univ. Tenn., Knoxville, TN; 4) Biomarin Pharmaceutical, Inc., Novato, CA.

Intrathecal recombinant human -L-iduronidase (IT rhIDU) given weekly in canine mucopolysaccharisosis I (MPS I) reduces central nervous system glycosaminoglycan (GAG) storage. Monthly, quarterly, and low-dose monthly IT rhIDU were tested to determine if less frequent IT injections were as effective. MPS I dogs received 1 mg of IT rhIDU once/month for 3-4 doses (monthly regimen, MR, n=4), 1 mg every 3 months for 3 doses (quarterly regimen, QR, n=4), or 0.46 mg once/month for 4 doses (low-dose regimen, LDR, n=2). Dogs also received weekly IV rhIDU. Iduronidase (IDU) levels reached 23-fold normal in brain, 7-fold in spinal cord, and 423-fold in meninges with MR, vs. 19-, 5-, and 264 -fold with QR. IDU levels were lower with LDR, at 3.4-fold normal in the brain (spinal results N/A). Brain GAG with MR was 4.000.596 g/mg dry weight (p=0.0008 vs. untreated), with QR 4.480.740 (p=0.0019), and with LDR 4.650.482 (p=0.02). These are similar to brain GAG levels in normal dogs (5.401.82, n=9) and roughly half the levels of untreated MPS I dogs (8.261.23, n=4). In the spinal cord, which has little storage, GAG levels were not significantly reduced (MR 4.050.62, p=0.18; QR 4.620.64, p=NS; LDR 5.030.91, p=NS; untreated 5.040.93, n=2; normal 3.020.71, n=4). GAG levels in the spinal meninges were reduced in all regimens by 61-70% (MR 13.94.13, p=0.003; QR 12.22.69, p=0.0006; LDR 10.62.09, p=0.01; untreated 35.93.03, n=2; normal 4.780.818, n=4). Brain pathology showed reduction of storage in MR and QR-treated dogs in neocortical meninges, perivascular spaces and neurons vs. untreated MPS I dogs (LDR results N/A). Pathology of the spinal cord and spinal meninges showed reduced GAG storage in MR, QR, and LDR-treated vs. untreated MPS I dogs. Some dogs developed mild-moderate lymphoplasmacytic meningitis. Monthly, quarterly, and low-dose monthly IT rhIDU may be effective alternatives to weekly treatment for canine MPS I.

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Molecular and cellular determinants of adenoviral gene therapy-associated toxicity. *V. Mane[1], G. Toietta[1], L. Medina-Kauwe[3], C. Clarke[1], L. Pastore[1], A.L. Beaudet[1], B. Lee[1,2].* 1) Dept Molec & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Cedars-Sinai Medical Center, UCLA, Los Angeles, CA.

Replication-deficient adenovirus (rAd) serves as an efficient gene therapy vector for liver directed gene transfer. Helper dependent vectors can carry up to 36 kb of therapeutic DNA and avoid cell mediated immune responses to viral proteins. Although they can mediate long term expression of both intracellular and secreted transgenes, e.g., urea cycle enzymes and clotting factors, they stimulate a robust innate immune response and acute toxicity after systemic delivery. By identifying host factors that contribute to this response, we may be able to decrease acute toxicity associated with a given vector dose.

By comparing rAd administration among several immunodeficient mouse strains, we found that TNF null mice exhibited decreased thrombocytopenia and decreased interleukin-6 production after rAd injection, compared to wild type mice. Importantly, mice pretreated with Enbrel, an FDA-approved inhibitor of TNF signaling, demonstrated a toxicity profile similar to null mice suggesting that pharmacological blockade of TNF has protective effects on rAd-induced acute toxicity.

Additionally, we addressed the cellular basis of host/vector interactions as a cause of the threshold effect, or nonlinear dose response, associated with systemic rAd delivery. Kupffer cells (KC) are liver macrophages whose location within the hepatic sinusoids facilitates engulfment of circulating vector particles. We used soluble adenoviral capsid proteins in an effort to block adenovirus-specific receptors on KC. Using this Kupffer cell saturation strategy, we demonstrated markedly increased transgene activity from low doses of rAd, compared to non-KC-saturated animals.

Our results suggest that transient modulation of inflammatory signals and cellular clearance mechanisms may lead to an improved therapeutic effect coupled to decreased toxicity of adenoviral gene therapy.

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Immune tolerance improves urinary GAG reduction during iduronidase replacement therapy in canine MPS I.

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To test whether immune responses to enzyme therapy might affect efficacy, we studied the effect that induction of immune tolerance in MPS I dogs had on efficacy of intravenous recombinant human -L-idurondiase (IV rhIDU) as measured by the degree of reduction in urinary glycosaminoglycans (GAG). 7 dogs with canine MPS I received oral cyclosporine and azathioprine combined with weekly low-dose (0.056 mg/kg) IV rhIDU (see Kakkis et al., PNAS 101(3):829-34, 2004). These dogs became tolerant to rhIDU (anti-iduronidase (IDU) antibody titer < 20 OD U/mL). 5 dogs received immune suppressive drugs in experimental tolerance regimens that failed to induce tolerance to rhIDU; these dogs had intermediate anti-IDU antibody titers (28.4-119 OD U/mL). All dogs were then treated with 0.58 mg/kg IV rhIDU weekly for 19-44 weeks. These were compared with 4 nontolerant (anti-IDU antibody titer 78.5-2295 OD U/mL) MPS I dogs receiving 0.58 mg/kg IV rhIDU alone for 9-14 weeks. The average reduction in urinary GAG excretion for tolerant dogs was 84.8 7.01%, vs. 60.0 11.9% reduction for nontolerant dogs (p =0.0017 vs. tolerant) and 53.4 11.8% for intermediate dogs (p=0.0002 vs. tolerant). Biochemical evaluation of kidney tissue was performed in 5 tolerant, 3 non-tolerant, and 5 intermediate dogs. IDU levels in tolerant dogs were higher, at 4.05 3.37 units/mg protein in the renal medulla and 12.2 10.5 in the cortex, than nontolerant: 1.30 0.865, p=0.23 medulla and 1.30 0.589, p=0.13 cortex; or intermediate: 0.916 0.390, p=0.07 medulla and 5.03 3.43, p=0.18 cortex. Renal GAG levels were lower, 11.8 1.85 g/mg dry weight medulla and 8.20 2.22 cortex in tolerant, than nontolerant: 21.7 13.1, p=0.07 medulla and 19.3 11.3, p=0.13 cortex, or intermediate: 25.6 5.70, p=0.0009 medulla and 19.3 3.16, p=0.0002 cortex. Immune tolerance to rhIDU can improve efficacy of IV rhIDU treatment as measured by urinary GAG excretion in canine MPS I, and this may be due to improved enzyme uptake or activity as reflected in greater GAG storage reduction in the kidney.

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Dramatic improvement of visual impairment in an MPS Is (Scheie) patient treated by laronidase. *V. Valayannopoulos[1], S. Hakiki[2], A. Chabli[3], K. Mention[1], C. Caillaud[4], JL. Duffier[2], M. Lemerrer[5], V. Cormier-Daire[5], P. de Lonlay[1], A. Munnich[5].* 1) Metabolic Ped & Neurology, Necker-Enfants Malades Hosp, Paris, France; 2) Ophthalmology Department,Necker-Enfants Malades Hosp, Paris, France; 3) Biochemistry B Department,Necker-Enfants Malades Hosp, Paris, France; 4) Biochemistry Department, Cochin Hosp, Paris, France; 5) Genetics Department,Necker-Enfants Malades Hosp, Paris, France.

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disorder due to alpha-L-iduronidase deficiency resulting in the storage in all tissues of glycosaminoglycans (GAG) responsible for a progressive, multisystemic disease. Enzyme replacement treatment (ERT) is now available (laronidase, recombinant human alpha-L-iduronidase). Although major improvements in sleep apnoeas and joint range of motion occurred in patients with the most severe symptoms, ERT has shown no significant efficacy on central and peripheral nervous system, eye and heart involvement. We report a 12 year old girl, presenting with MPS I (Scheie phenotype): restricted mobility, carpal tunnel syndrome, a slightly coarse face, hearing loss with tinnitus, cardiac involvement with mitral valve prolapse but no mental retardation. This patient suffered an acute unilateral visual loss, related to a left optic nerve compression by mucopolysaccharide infiltration shown by B-mode ultrasonography. Visual field assessment revealed a superior nasal defect. The patient was started on laronidase 100 U/kg weekly infusions. Visual acuity and visual field impairment dramatically improved within six months of treatment. This improvement has persisted so far after 18 months of treatment. Moreover she showed improved joint mobility («able to comb her hair »), better physical condition and improved hearing with no tinnitus. No adverse effects occurred during the infusions. In conclusion, this case report illustrates a unique efficacy of ERT in MPS retinal involvement, despite several previous reports of unsuccessful treatment of this condition.

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Impact of long term Aldurazyme (laronidase) replacement therapy on cardiac disease progression in MPS I patients. *M. Sifuentes[1], R. Doroshow[2], R. Hoft[1], G. Mason[1], J. Phillips[1], I. Walot[1], M. Diament[1], S. Okazaki[1], K. Huff[1], G. Cox[3], B. Lyons[4], S. Swiedler[4], E. Kakkis[4].* 1) Dept of Pediatrics, Harbor-UCLA Medical Center, Torrance CA; 2) Children's National Medical Center, Washington DC; 3) Genzyme Corp, Cambridge MA; 4) BioMarin Pharmaceutical Inc, Novato CA.

In mucopolysaccharidosis I (MPS I), accumulation of partially degraded glycosaminoglycans (GAG) occurs in a variety of tissues. Cardiovascular lesions occur across the disease spectrum and progressively worsen often leading to death in many patients (pts) by the second or third decade. Six years (yr) follow-up data from 5 MPS I pts (4 Hurler-Scheie, 1 Scheie) assessed in an observational study were reviewed to evaluate the long term effects of Aldurazyme treatment on the cardiac status. A cardiac function scoring system was devised that combined data from the NYHA classification, ECG, echocardiogram, chest X-ray, and the cardiology physical exam to consolidate the wide ranging changes into a single score. The mean age of the 5 pts at follow-up was 18.4 yrs (range 14-24 yrs). At pretreatment, all pts had abnormal functional capacity (NYHA Class II (n=3), III (n=2)). By 1 yr of treatment, 3 had improved to NYHA Class I and by 6 yrs, 4 had improved to NYHA Class I. The cardiac function score (a higher score represents more disease) reduced from 11.5 (mean; range[8- 17]) at pretreatment to 9.2 (mean; range [5-12]) at Yr 6. Three pts had increases in score from pretreatment, ranging from 0.5 points to 1.5 points, and 2 pts had decreases of 1.5 points and 12 points, respectively. A slight increase in valvular regurgitation scores was noted from week 52 to Yr 6. At Yr 6 cardiomegaly assessed on X- ray remained stable in 2 pts worsened in 1 and improve in 2 patients. After 6 yrs of Aldurazyme, cardiac function remained stable or slightly deteriorated in a phenotype and age group where important cardiac disease progression was expected. Increases in endurance reflected by the NYHA classification most likely represent improvement across many organ systems, including the heart. Longer follow-up is required to assess if the impact of therapy on cardiac function scores translate in increased life expectancy.

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Dose-response assessment in Gaucher patients on enzyme replacement therapy: long-term monitoring with ^{99m}Tc-Sestamibi scintigraphy. *G. Mariani[1], P.A. Erba[1], F. Minichilli[2], A. Pierini[2], F. Giona[3], A. Amendola[3], M. Filocamo[4], F. Buffoni[5], R.O. Brady[6].* 1) Dept. of Nuclear Medicine, University of Pisa Medical School, Pisa, Italy; 2) CNR Institute of Clinical Physiology, Pisa, Italy; 3) Dept. of Cell Biotechnologies and Hematology, University of Rome La Sapienza, Rome, Italy; 4) G. Gaslini Childrens Hospital, Genoa, Italy; 5) Nuclear Medicine Service, Town Hospital, Leghorn, Italy; 6) Developmental and Metabolic Neurology Branch, NINDS, NIH, Bethesda, MD, USA.

Skeletal responses to enzyme replacement therapy (ERT) in Gaucher pts occur slowly. Assessment of the skeletal component of Gaucher disease for monitoring efficacy of ERT over time is problematic. ^{99m}Tc-Sestamibi scintigraphy provides a score of bone marrow infiltration closely correlated with overall severity of Gaucher disease. We determined the utility of such score to monitor ERT efficacy in Gaucher pts. 48 Gaucher pts underwent a baseline ^{99m}Tc-Sestamibi scan, then again 10-134 months later (mean 49.7). Statistical analysis was based on the stepwise backward and cross sectional time series approach. More pts on ERT reached normal scintigraphic score when clinical disease duration was <10 yr (33.3% versus 5.6%, p=0.03-0.049), and more pts whose age was <20 yr reached normal score on ERT (42.8% versus 8%, p=0.03-0.07). Improvement in the scintigraphic score was correlated with ERT dose/month (p=0.011-0.047), ERT duration (p=0.017) and ERT cumulative dose (p=0.014-0.047). The mean dose/month inducing improvement in the score (36.852.94 U/kg per month) was significantly higher than in pts who did not improve on ERT (24.044.92 U/kg, p=0.004), as also higher was the cumulative ERT dose (878.8168.5 U/kg vs 495.5154.6 U/kg, p=0.0112). The ^{99m}Tc-Sestamibi score is an excellent predictor of response to ERT in Gaucher pts. Score changes are more closely correlated with the ERT variables than other clinical scores. Better score responses to ERT are expected in younger pts and in pts with shorter clinical disease duration. A dose-response relationship was identified between ERT and scintigraphic score. The minimum amount of enzyme required to improve the scintigraphic score was defined.

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IMPROVEMENT OF SYMPATHETIC SKIN RESPONSES AFTER ENZYME REPLACEMENT THERAPY IN FABRY DISEASE. *C.B.O. Netto[1], L.B. Jardim[1,2], J. Becker[2], D. Nora[2], U. Matte[1], F. Pereira[1], M. Burin[1], I. Gomes[2], R. Giugliani[1].* 1) Genetics, UFRGS-HCPA, Porto Alegre, Porto Alegre, Brazil; 2) Neurology, UFRGS-HCPA, Porto Alegre,Brazil.

Introduction and Objectives:Fabry disease is an X-linked disorder caused by deficient activity of the lisosomal enzyme alfa-galactosidase A. We report the effect of 24 months of enzyme replacement therapy (ERT), in sympathetic skin responses (SSR) of patients with FD. Patients and methods: 7 male patients from 4 families were included in an open-label protocol using agalsidase-alpha. Two main measurements were done at baseline, and one and two years after the start of ERT: (1) a standard neurological examination (NE); (2) and SSR amplitudes. Results: in the 2nd year of ERT, there was a general improvement in the subjective reports of acroparesthesias, episodes of diarrhea and reduced sweating. There were no significant differences between NE in this time period, although thermal perception tended to worse. Before the start of ERT, SSR amplitudes were either too small or even absent (4/7 patients): the average (range) amplitude of 122 mV (0 to 492) was statistically smaller than those found in control group - 1453.6 mV (619.7 to 2754) ($p < 0.0001$, t test). Mean sd SSR amplitude aroused to 683 442, in the 1st year, and to 1088 690 mV, in the 2nd year of ERT, reaching the range found in normal, control group. Discussion: ERT continuously improved sympathetic function in Fabry patients, measured by SSR, in 2 years of observation. Although the mechanism of SSR improvement is unknown, this response to ERT can be clinically significant, if this is reflecting a normalisation of sweating, and, perhaps, of the body thermal control, with an expected reduction in acroparesthesias. Support: TKT.

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Aldurazyme therapy in children less than 5 years of age with MPS I:preliminary data on urinary GAG and antibodies. *E. Wraith[1], N. Guffon[2], A. Van der Ploeg[3], M. Beck[4].* 1) Royal Manchester Children Hosp, Manchester, United Kingdom; 2) Hôpital E Herriot, Lyon, France; 3) Sophia Childrens Hospital, Rotterdam, the Netherlands; 4) Children`s Hospital, University of Mainz, Germany.

It has been postulated that patients with severe mucopolysaccharidosis I (MPS I) patients and no residual -L-iduronidase would be more likely to develop a strong immunologic response to treatment with Aldurazyme (laronidase, recombinant human -L-iduronidase) that might impair the clearance of GAG and lead to more adverse events. An open-label, 52 wk study of the safety and efficacy of Aldurazyme in 20 MPS I patients less than 5 yrs of age was recently carried out. As part of this study, antibody (IgG) titers to Aldurazyme and urinary GAG (uGAG) levels were measured. Patients initially received Aldurazyme at the label dose of 0.58 mg/kg (100 U/kg) IV once weekly. At Wk 26, 4 patients had their dose doubled to 1.16 mg/kg because of uGAG levels that remained above 200 mg/g creatinine at Wk 22. Adverse events were monitored throughout the study. The mean age of patients at baseline was 35 months (range 6-62 months). Of the 20 patients, 16 were classified as Hurler and 4 as Hurler-Scheie. Most patients (n=18) developed IgG antibodies to laronidase by Wk 4. The maximum antibody titer was 1/204800. Peak antibody titers occurred between Wk 4 and 20 and then began to decrease in some patients after Wk 12. The mean uGAG level at baseline (n=19) was 569 mg/g creatinine (sd 174.3; range: 211,5 - 926,7). The mean decrease in uGAG level was 56.3%; (sd 13.2%) (n=19) after 26 wks therapy with Aldurazyme. A similar reduction was observed after 52 wks in the first 10 patients with available data. Preliminary safety data suggests that Aldurazyme appears to be well-tolerated by these young patients, including those who received the 1.16 mg/kg dose. Final analysis of the complete dataset is currently in progress.

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Long-term improvement in joint range of motion in mucopolysaccharidosis I (MPS I) patients treated with Aldurazyme (laronidase) in a Phase 3 extension study. *G. Pastores[1], J. Muenzer[2], M. Beck[3], L. Clarke[4], E. Wraith[5].*
1) Neurology, New York University School of Medicine, New York; 2) Pediatrics, University of North Carolina, Chapel Hill, NC; 3) Pediatrics, Johannes Gutenberg Universität, Mainz, Germany; 4) Medical Genetics, University of British Columbia, Vancouver, Canada; 5) Willink Biochemical Genetics Unit, Royal Manchester Childrens Hospital, Manchester, UK.

All patients from a 26-week randomized, double-blind, placebo-controlled study with Aldurazyme (laronidase-rhIDU) were enrolled into an open-label study. Patients crossing over from placebo to rhIDU (N=23; total exposure 144 weeks) were designated placebo/rhIDU, the others (N=22; total exposure 170 weeks) were designated rhIDU/rhIDU. Active flexion and extension of left and right shoulders and knees was measured. Two patients with symptomatic cervical cord compression were excluded from this analysis. Mean shoulder flexion in the rhIDU/rhIDU group gradually improved by 4.3 degrees, 8.7 degrees, 12.4 degrees, and 13.7 degrees at 50, 62, 86, and 110 weeks. At Week 170, the mean improvement in shoulder flexion was 15.5 degrees in this group. Patients in the placebo/rhIDU group demonstrated a mean improvement of 14.5 degrees by Week 144. A similar pattern of improvement was seen in the other joint range of motion measurements. Mean shoulder extension in the rhIDU/rhIDU group gradually improved by 4.6 degrees, 6.0 degrees, 7.1 degrees, 9.6 degrees at 50, 62, 86, and 110 weeks. At Week 170, the mean improvement in shoulder extension was 12.7 degrees, and in the placebo/rhIDU group the mean improvement at week 144 was 10.0 degrees. Mean knee flexion and mean knee extension improved in the rhIDU/rhIDU group by 13.4 degrees and 8.5 degrees. In the placebo/rhIDU group, the improvements amounted to 5.1 degrees and 4.0 degrees. Approximately 50% of the patients experienced infusion related events, the majority of which did not require treatment. Continued improvements in joint range of motion have been observed over the first three years of treatment. These changes are expected to translate into increased abilities to carry out activities of daily living.

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Children with Fabry disease reveal the importance of very early treatment. *C.P. Lorentz[1], R.J. Desnick[2], C.B. Whitley[1].* 1) University of Minnesota, Minneapolis, MN; 2) Mt. Sinai School of Medicine, New York, NY.

Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of alpha-galactosidase A. Clinical onset in childhood includes debilitating acroparesthesias and excruciating pain crises, hypohidrosis, and gastrointestinal problems. With advancing age, patients develop renal failure, cardiac disease and strokes leading to early demise. Double-blind randomized placebo-controlled trials in adults of enzyme replacement therapy (ERT) with Fabrazyme at 1 mg/kg have shown clearance of the accumulated globotriaosylceramide (GL-3) in the plasma, vascular endothelium, heart, liver, skin, and kidney. However, limited experience is available on the effects of ERT in children with Fabry disease. Here we report the response to Fabrazyme treatment (1 mg/kg every 2 weeks) in two affected boys aged 4 and 11/12 yr (Patient 1; genotype I270T) and 12 and 1/6 yr (Patient 2; genotype Y216D). Patient 1 had episodes of acrimonious hand-shaking, abdominal cramping, and 6 to 10 bowel movements daily. After 6 weeks of therapy, there was a dramatic resolution of abdominal pain and diarrhea. Plasma GL-3 decreased from 10.6 to 5.6 mg/L (normal <7 mg/L). Patient 2 also complained of frequent abdominal cramping and diarrhea, episodic acroparesthesias of the hands and feet, and heat intolerance due to hypohidrosis. His acroparesthesias stopped after 6 weeks of treatment; by 6 months, his sweating was normal, and he was able to play 9 innings of baseball in warm weather for the first time. His plasma GL-3 declined from 8.7 to 2.4 mg/L. In these children, the acroparesthesias, hypohidrosis, and gastrointestinal manifestations, the early severe, debilitating symptoms of Fabry disease that impaired their quality of life, were resolved by ERT. Thus, ERT in affected boys and symptomatic heterozygous females is recommended to prevent and/or treat the early disease symptoms and to prevent permanent, irreversible disease damage. Fabry disease should be treated as early as possible and especially in childhood, as recommended by a recent expert consensus article (Desnick et al., Ann Intern Med 138:338, 2003).

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KIDNEY FUNCTION EVALUATION AFTER 24-MONTHS OF ENZYME REPLACEMENT THERAPY FOR FABRY DISEASE. *E. Barros[1], C.B.O. Netto[2], C. Cecchin[2], M. Burin[2], S. Brustolin[2], L. Jardim[1,2], R. Giugliani[2].* 1) Internal Medicine, UFRGS, Porto Alegre, RS, Brazil; 2) Genetics, UFRGS-HCPA, Porto Alegre,Brazil.

INTRODUCTION AND AIMS: Fabry disease (FD) is X-linked metabolic disorder due to the deficiency of alfa-galacosidase A. We report our experience after 2 years of ERT concerning the kidney function. METHODS AND PATIENTS: Nine FD patients (7 males) were included in an open-label protocol using agalsidase-alpha..We measured the following outcomes at baseline, 12 and 24 months of ERT: urinary protein, glomerular filtration rate (GFR), plasma creatinine, and the estimated creatinine endogenous clearance (CEC). RESULTS: Non-nephrotic proteinuria was present in 5/9, 3/8 and 3/6 patients at baseline, 12 and 24 months of ERT, respectively. The mean (sd) of the following variables, obtained at the same time points were: 105 (38), 116 (39) and 105 (37) ml/min for the GFR; 0.8 (0.5), 0.7 (0.1) and 0.8 (0.05) mg% for plasma creatinine; and 127 (40), 125 (24) and 102 (12) ml/min, for estimated CEC. At baseline, two patients were at the beginning of their renal diseases: patient four, age 28, progressed to end-stage renal disease and had kidney transplantation by the 12th month; and patient one, age 46, who in spite of ERT, continued to lose renal function by around 12 ml/min/. CONCLUSIONS: non-nephrotic proteinuria was found in the same proportions as those already reported in studies on the natural history of FD, and showed no changes during the 2 years of ERT. Only two patients of the present series started their ERT with renal disease already established, and therefore, only they could be compared with historical controls of FD without treatment. We conclude that adequate follow up is required to judge ERT effects over kidney, especially because our patients were still young, and/or free of renal dysfunctions. Support: TKT.

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Successful reinstitution of agalsidase beta therapy in patients with previous IgE antibody production or positive skin testing. *D.C. Bodensteiner[1], D.P. Germain[2], C.R. Scott[3], K.B. Sims[4].* 1) U Kansas Medical Center, Kansas City, KS; 2) Hôpital Européen George Pompidou, Paris, France; 3) U Washington School of Medicine, Seattle, WA; 4) Massachusetts General Hospital, Boston, MA.

Enzyme replacement therapy (ERT) with 1 mg/kg of recombinant human -galactosidase A (agalsidase beta; Fabrazyme) administered intravenously every 2 weeks is a safe and effective treatment for Fabry disease. Of 169 patients treated with agalsidase beta in clinical studies, 7 developed serum IgE antibodies or had a positive skin test to agalsidase beta. A rechallenge protocol was tested in a multicenter, open-label, Phase 2 study to determine if ERT with agalsidase beta could be safely reinstituted in these patients. ERT was performed under supervision of the investigator and a qualified allergist. Infusions 1 and 2 were administered a week apart at 0.5 mg/kg; the initial infusion rate of 0.01 mg/min could be doubled every 30 min up to 0.25 mg/min if no significant infusion-associated reactions (IARs) occurred. After infusion 2, a dose of 1.0 mg/kg every 2 weeks was allowed at infusion rates that could be progressively increased, but total infusion duration had to be 2 hr. For infusions 1 through 4, pretreatment medication was prohibited so IARs could be readily identified. Subsequently, antihistamines, antipyretics, -agonists, and steroids were permitted for managing IARs. Patients were continuously monitored for adverse events (AEs) during and up to 2 hr after infusions. Among the 6 patients enrolled, 5 successfully received agalsidase beta at doses of 0.5 to 1.0 mg/kg; 1 withdrew voluntarily due to IAR recurrence. There were no reports of anaphylaxis. All AEs were of mild or moderate severity, with IARs accounting for most AEs. Four serious AEs, all considered IARs, occurred in 3 patients and were managed by infusion rate reductions and medication. Urticaria, the most common IAR, was not associated with histamine release and appeared to be complement-mediated. Hence, agalsidase beta therapy can be safely reinstituted in patients with positive skin testing or IgE antibody production using a graded approach to dose and infusion rate, medication to mitigate IARs, and careful patient monitoring.

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Efficacy of reduced-dose alglucerase therapy in gaucher disease. *A.F. Moore[1], H. Zhu[2], D. Kuter[1].* 1) Internal Medicine, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Massachusetts General Hospital, Boston, MA.

INTRODUCTION: Gaucher disease is a lysosomal storage disorder caused by decreased glucocerebrosidase activity. Enzyme replacement therapy with alglucerase is efficacious in treating Gaucher Disease at a cost of over $500,000 annually. This cost has prompted interest in finding the lowest effective dose; however, there are few studies of long term, reduced-dose therapy. This paper describes the 10-year experience of 15 patients on reduced-dose alglucerase therapy (30 U/kg every 2 weeks) at the Massachusetts General Hospital (MGH). OBJECTIVES: To evaluate the efficacy of reduced-dose alglucerase therapy. SUBJECTS AND METHODS: Data over 10 years was recorded at MGH for 15 patients. Response was measured with annual measurements of hematocrit, platelet counts, angiotensin-converting enzyme (ACE) levels, ferritin levels, spleen and liver size, and bone crisis frequency. Body mass index (BMI) was calculated from serial height and weight measurements. RESULTS: Patients treated with reduced-dose alglucerase therapy demonstrated clinically significant increases in hematocrit and platelet count. Most anemic patients achieved a 6 percent increase in hematocrit within 4 years of initiating therapy, and a majority of patients normalized their platelet counts within 2 years. During the final 3 years of the study, both the average hematocrit and platelet counts decreased but never returned to initial values. ACE and ferritin levels, biologic markers of Gaucher disease activity, returned to normal within 1 year of initiating therapy and remained normal for the remainder of the study. Organ size was measured by computed tomography volumetric reconstruction of 2 patients, both of whom demonstrated reduction of initial organomegaly. Patients gained weight during therapy with 29% increasing their BMIs at least 20%. CONCLUSION: Gaucher disease can be effectively treated with low-dose alglucerase therapy at a dose of 30 U/kg every 2 weeks (60 U/kg/month) with similar efficacy as other higher-dose regimens for the initial 5 to 7 years of therapy. Some attenuation in response may occur when patients are treated longer.

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Dose responsive toxicological findings following intravenous administration of recombinant human acid sphingomyelinase(rhASM) to acid sphingomyelinase knock-out(ASMKO) mice. *C. Nickerson, J. Murray, A. Vitsky, M. Hawes, S. Ryan, P. Ewing, B.L. Thurberg, L. Andrews.* Genzyme Corporation, Framingham, MA.

ASM is a lysosomal enzyme that cleaves sphingomyelin into ceramide and phosphorlycholine. ASM deficiency results in the allelic disorders known as Niemann-Pick disease(NPD) Types A and B. The ASMKO mouse is being used for the evaluation of the pharmacodynamic effects of rhASM and the development of rhASM as a potential enzyme replacement therapy for NPD. While single dose and chronic repeated dose toxicity studies conducted in normal animals have shown no adverse effects from rhASM administration up to the highest doses administered, the ASMKO mouse has been shown to be a sensitive model for the evaluation of the toxicity of rhASM. Single dose toxicity studies show dose responsive histopathological findings in the liver and adrenals of ASMKO mice when compared to vehicle, with no significant adverse effects in the C57BL/6 mice. Findings at low doses included randomly scattered hepatocellular ballooning degeneration and apoptosis as well as mild adrenocortical necrosis. Lesions in the adrenal and kidney at high doses correlate with systemic effects of hypotension and shock. These results suggest the toxicity is related to the substrate or substrate degradation products. In a repeated dose toxicity study, ASMKO mice received rhASM every other week for 12 weeks, with a four week recovery. Histopathological findings in the liver and adrenal were similar to single dose studies though changes were milder and reversible. Single or repeated therapeutic doses of rhASM that reduce substrate levels, followed by a known toxic dose, results in the reduction or prevention of the toxicity. These data suggest the toxicity is associated with the Cmax of rhASM and the amount and rate of substrate degradation. Results of these studies in normal animals and transgenic mice demonstrate the utility of including animal models of disease in the toxicological evaluation of potential therapeutics. Additional studies are underway to further characterize the molecular basis of toxicity in ASMKO mice and to identify potential safety biomarkers for use in human clinical trials of rhASM.

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Testing of new therapeutic interventions in Fanconi Anemia. *M. Grompe[1], M. Noll[1], S. Houghtaling[1], Y. Akkari[1], M. Finegold[2], S. Olson[1], M. Kay[3].* 1) Dept Molec/Med Genetics, Oregon Health & Sci Univ, Portland, OR; 2) Baylor Coll of Med, Houston, TX; 3) Stanford Univ., Palo Alto, CA.

The life span of patients with Fanconi Anemia (FA) is severely shortened by bone marrow failure and malignancies and currently, allogeneic bone-marrow transplantation is the only therapeutic option. We are using mouse models of FA to preclinically test various therapeutic interventions. First, non-viral methods for gene transfer into hematopoietic stem cells (HSC) are being tested. In FA retroviral vector gene therapy is hampered by the requirement for prolonged ex vivo manipulation and the scarcity of HSC. We therefore utilized the non-viral Sleeping Beauty (SB) transposon system to facilitate stable, gene transfer of the human FANCC cDNA without ex vivo culture. This was achieved either by electroporation of naked DNA into whole bone marrow or by direct injection into the femur cavity. We show that a) HSC from Fancc -/- mice can be transduced by plasmid DNA; b) stable transposition of plasmid DNA into the genome of HSC is possible and c) long-term therapeutic benefit could be achieved using this method. Non-viral gene transfer may be a viable alternative for the treatment of hematopoietic diseases. Second, methods to reduce the number of solid tumors in FA patients are being tested.. Fancd2-/-p53+/- mice develop epithelial tumors at about one year of age. We are using this strain of mice to test the efficacy of drugs which may have the potential to delay tumor formation. Recently, it was shown that the superoxide dismutase mimetic drug tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) can significantly delay tumor onset in a murine model of telangiectasia. It is thought that tempol reduces DNA damage induced by free oxygen radicals and thereby ameliorates tumor formation. Oxidative damage also plays a role in FA oncogenesis. We have generated 40 female Fancd2-/-p53+/- mice, which have a 50% expected rate of epithelial tumor formation by age 15 months. Half of these animals are being treated with a control diet and half are being given tempol. At an average age of ~9 months, significant numbers of tumors have not yet been observed in either the treated or control mice. Data for an average treatment duration of 14 months will be presented.

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Construction of a novel human artificial chromosome (HAC) and its use for gene delivery. *M. Oshimura[1, 2, 3], R. Xianying[2], A. Otsuki[1], M. Kakeda[4], M. Hiratsuka[2], H. Yamada[1], T. Suda[1], Y. Kazuki[1], M. Nishikawa[4], K. Tomizuka[4], M. Kawahara[5], T. Nagamune[5], M. Katoh[3], T. Inoue[3], A. Kurimasa[1].* 1) Dept. Biomed. Sci., Inst. Regenerative Med. and Biofunction; 2) Dept. of Mol. & Cell Genet; 3) Dept. Hum. Genome Sci., Grad. Sch. Med. Sci., Tottori Univ., Yonago, Tottori, JAPAN,; 4) Pharm. Res. Lab., Pharm. Div., Kirin Brewery Co., Ltd. Takasaki, Gunma, JAPAN,; 5) Dept. Chem. & Biotech., Grad. Sch. of Engineering, The Univ. of Tokyo, Hongo, Bunkyo-ku, Tokyo, JAPAN.

Ex vivo gene delivery followed by autologous transplantation is a way for gene therapy and regenerative medicine. Prerequisites for gene delivery vectors are 1) long term stable maintenance in host cells, 2) no risk for disrupting host genome by integration and 3) appropriate regulation of transgene in host cells. Previously, transfer of engineered human chromosome to mouse ES cells via microcell fusion achieved production of mice expressing functional human immunoglobulin, suggesting the potential use of chromosome vector. Thus, we addressed the feasibility of constructing chromosome-based ex vivo gene delivery system. We have developed a structurally defined HAC into which circular DNA can be loaded by a Cre/loxP-system. In chicken DT40 cells, distal q- and p-arm were truncated by telomere seeding from the sequence-defined human chromosome 21, and a loxP site was introduced. These 21HACs were transferable and stably maintained in human HT1080 and mouse ES cells. The EGFP gene on the HAC was expressed persistently in these cells, indicating that the 21HAC might be useful for functional study of transgene in vitro. Progress in testing the performance of the HAC vector will be reported; 1) tetracycline-inducible expression of DNA-Pkcs gene, 2) induction of tissue-specific expression of EGFP reporter gene accompanying in vitro differentiation in human Mesenchymal Stem Cells, 3) construction of non-beta cells capable of inducible expression of insulin, 4) persistent expression of erythropoietin gene in normal human fibroblasts, 5) exogenous gene expression and antigen-mediated growth regulation of human hematopoietic cells.

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Development of therapeutic siRNAs that specifically target keratin 6a for treatment of the skin disorder pachyonychia congenita. *R. Kaspar[1], F. Smith[2], W. McLean[2], M. Landthaler[3], R. Leube[4], R. Hickerson[1].* 1) TransDerm, Santa Cruz, CA; 2) Ninewells Medical School, Dundee, UK; 3) Rockefeller University, NY; 4) Johannnes Gutenberg-Universität, Mainz.

The discovery that siRNAs silence gene expression without inducing an immune response has resulted in intense therapeutic development efforts. Due to recent advances in nucleic acid skin delivery, siRNA offers a novel approach for treating skin disorders. Pachyonychia congenita (PC) is a rare autosomal dominant disorder that results in hypertrophic nail dystrophy and focal palmoplantar keratoderma with blisters. Two types of PC have been described: PC-1 is caused by mutations in genes encoding keratin (K) 6a or K16 genes, PC-2 from mutations in K6b or K17. In K6a, there are several recurrent mutations at the N171 site, which is either deleted or a single nucleotide (nt) is mutated resulting in an amino acid change (e.g. N171K). We show that a fusion protein consisting of K6a and YFP reporter (K6a-wt/YFP) results in keratin filaments in transfected PLC cells as assayed by fluorescence microscopy. A similar construct with a PC K6a N171K mutation results in aggregate formation and filament disruption. To investigate whether siRNAs can rescue the aberrant filaments, siRNAs were designed to target the N171K mutation. Co-transfection of the K6a-YFP constructs (wt or N171K) and siRNAs into 293 cells revealed inhibitors that preferentially target mutant K6a mRNA (e.g. one siRNA had IC50 values of 0.6 nM and 0.1 nM against wt and mutant constructs, respectively). Due to the strong sequence homology between K6a and K6b (and the apparent functional redundancy of K6a in knockout mice), we designed inhibitors that target unique regions of K6a, specifically in the 3UTR. Co-transfection experiments with the K6a-wt/YFP construct and K6a 3UTR-specific siRNA inhibitors showed decreased K6a-wt/YFP expression (IC50 <0.2 nM). These studies suggest that siRNAs developed against K6a in general, or PC-specific mutations, may be effective PC therapeutics and further suggest that designer siRNAs may allow effective treatment of a host of genetic skin disorders and other tissues using a tailored approach.

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Nonviral gene transfer in the Mucopolysaccharidosis I murine model. *U. Matte[1], M. Camassola[1,2], L.M. Braga[2], A.D. Canedo[2], T.P. Dalberto[2], M. Burin[3], N. Nardi[2], R. Giugliani[1,3].* 1) Center for Gene Therapy, HCPA, Porto Alegre, RS, Brazil; 2) Genetics Department, UFRGS, Porto Alegre, RS, Brazil; 3) Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil.

Mucopolysaccharidosis I (MPS I) is a lysosomal disorder characterized by a deficiency of the enzyme alpha-L-iduronidase (IDUA), that leads to the accumulation of glycosaminoglycans (GAGs). Presently, available treatments include bone marrow transplantation and enzyme replacement therapies, both of which are limited in their effects. In this work, knockout (KO) MPS I mice were treated with a nonviral vector containing the human IDUA cDNA. KO mice were transfected by hydrodynamic injection of pRIDUA in the caudal vein (iv, n = 3) or by intraperitoneal injection of pRIDUA/Superfect complexes (ip, n = 3). GAG level and IDUA activity were analyzed in the kidneys, spleen, lungs, brain and liver. The expression of IDUA in the organs of iv- and ip-treated mice was also analyzed by real-time reverse transcription (RT) PCR and compared by relative quantification. The concentration of GAGs in the organs was different between KO and wild-type mice. In the spleen and liver, GAG levels were lower in iv- and ip-treated KO mice than in control nontreated animals. Real-time RT-PCR showed that the transgene is expressed in all the analyzed organs of ip- and iv-treated KO mice. Enzyme activity was similarly observed in all the organs analyzed. Our data suggest that this kind of transfection may be a useful tool for studies of nonviral protocols for gene therapy of MPS. (Finacial support: CNPq, FAPERGS, FIPE-HCPA).

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High Efficiency Hepatic Transduction with Minimal Toxicity Following Hydrodynamic Injection of HDAd into Nonhuman Primates. *N. Brunetti-Pierri[1], C. Mullins[2], G.E. Stapleton[2], D. Palmer[1], M. Finegold[3], K. Rice[4], D. Carey[4], A.L. Beaudet[1], P. Ng[1].* 1) Dept Molec & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics Cardiology, Baylor College of Medidicine, Houston, TX; 3) Dept. of Pathology,Baylor College of Medidicine, Houston, TX; 4) Southwest Foundation for Biomedical Research, San Antonio, TX.

Helper-dependent adenoviral vectors (HDAd) are attractive vectors for liver-directed gene therapy because they can mediate sustained, high level transgene expression with no long-term toxicity. However, as a consequence of a threshold effect, high vector doses are required to achieve efficient hepatic transduction by peripheral intravenous injection which results in a dose-dependent activation of the innate inflammatory response. Systemic hydrodynamic injection of HDAd into mice resulted in exceedingly high levels of hepatic transduction and reduction in both systemic vector dissemination and acute proinflammatory cytokines compared to conventional injection. Hydrodynamic injection cannot be applied to larger animals due to the large injection volume. We have developed a minimally invasive method to mimic hydrodynamic injection in nonhuman primates which does not require injection of large volumes. This method involves the use of balloon occlusion catheters percutaneously positioned in the inferior vena cava to transiently obstruct hepatic venous outflow to increase the intrahepatic pressure prior to simple peripheral intravenous injection of HDAd in a small volume. We demonstrate that this novel method of vector delivery results in an exceedingly high and unprecedented level of hepatic transduction with low vector doses resulting in stable, long-term transgene expression. The procedure itself was uneventful and was accompanied by minimal evidence of liver injury and minimal, transient elevations of proinflammatory cytokines. This novel method of delivering HDAd is simple, minimally invasive, and clinically relevant since it increases the therapeutic index of HDAd for liver-directed gene therapy by allowing high efficiency hepatic transduction with low vector doses and with negligible acute or chronic toxicities.

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High Efficiency Hepatic Transduction and Long-Term Transgene Expression by Delivering Helper-Dependent Adenoviral Vectors into the Surgically Isolated Liver of Nonhuman Primates. *D.J. Palmer[1], N. Brunetti-Pierri[1], T. Ng[2], D. Iannitti[2], W. Cioffi[2], M. Finegold[3], K. Rice[4], D. Carey[4], A.L. Beaudet[1], P. Ng[1].* 1) Dept. Mol & Human Genetics, Baylor College of Medicine, Houston,TX; 2) Department of Surgery, Brown Medical School, Providence, RI; 3) Dept. Pathology, Baylor College of Medicine, Houston,TX; 4) Southwest Foundation for Biomedical Research, San Antonio,TX.

Helper-dependent adenoviral vectors (HDAd) are attractive vectors for liver-directed gene therapy because they can mediate long-term, high level transgene expression with no long-term toxicity. However, as a consequence of a threshold effect, high vector doses are required to achieve efficient hepatic transduction by peripheral intravenous injection which unfortunately results in a dose-dependent activation of the innate inflammatory response. Strategies to overcome the threshold to efficient hepatic transduction are needed to improve the therapeutic index of HDAd. We hypothesized that this obstacle could be surmounted by delivering the vector exclusively to the liver. To test this hypothesis, we have injected HDAd directly into surgically isolated livers via the portal vein in nonhuman primates. Total hepatic isolation was achieved by occluding both hepatic inflow and outflow. Prior to total hepatic isolation, saline was infused into the portal vein to flush blood out of the liver. The vector was then injected into the liver via the portal vein and allowed to dwell for 30 minutes, following which unabsorbed vector was flushed out via a catheter placed in the vena cava to minimize systemic vector dissemination. Our results revealed that significantly higher hepatic transduction efficiencies can be achieved with relatively low vector doses compared to peripheral intravenous injection. Importantly, stable, high levels of transgene expression were observed for up to one year with no long-term toxicity. This approach may increase the safety and efficacy of HDAd-mediated, liver-directed gene therapy by minimizing the dose required to achieve efficient hepatic transduction and by minimizing systemic vector dissemination.

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Aerosol delivery of helper-dependent adenoviral vector into nonhuman primate lungs results in high efficiency pulmonary transduction with minimal toxicity. *P. Ng[1], N. Brunetti-Pierri[1], D. Koehler[2], R. McConnell[3], J. Katkin[3], D. Palmer[1], D. Dimmock[1], J. Hu[2], M. Finegold[4], D. Carey[5], K. Rice[5], A.L. Beaudet[1], P. Hiatt[3].* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Programme in Lung Biology Research and the Canadian Institutes of Health Research Group in Lung Development, Hospital for Sick Children, Toronto; 3) Dept of Pediatrics, Baylor Col Medicine, Houston, TX; 4) Dept Pathology, Baylor Col Medicine, Houston, TX; 5) Southwest Foundation for Biomedical Research, San Antonio, TX.

Cystic fibrosis is caused by recessive mutations in a membrane chloride channel gene (CFTR) expressed in the airway epithelium and is characterized by persistent, progressive inflammation and infection, eventually leading to lung failure. A major obstacle to CF gene therapy is inefficient pulmonary transduction by viral and nonviral vectors in large animals and humans. To address this obstacle, we have used a novel strategy for efficient aerosol delivery into nonhuman primate lungs of a helper-dependent adenoviral vector (HDAd) bearing a human airway epithelial cell and submucosal gland cell-specific expression cassette driving either LacZ or bAFP. The AeroProbe, an intracorporeal nebulizing catheter, was used to aerosolize HDAd formulated in 0.1% L--lysophosphatidylcholine directly into the airways of nonhuman primates. Extensive and unprecedented high levels of transduction of the ciliated epithelium of the trachea, bronchi and bronchioles as well as submucosal glands were observed. None of the animals treated showed signs of respiratory distress or chest X-rays abnormalities. Laboratory findings (chemistries, cell counts, cytokines, etc) in the blood and BAL fluid were unremarkable. This study demonstrates a novel and promising strategy to achieve high efficiency transduction of the trachea, the proximal and distal airway epithelium and submucosal glands in a large animal model for HDAd-mediated, lung-directed gene therapy for CF.

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Development of long-term treatment for hypertension using human skin gene therapy to deliver systemic Atrial Natriuretic Peptide (ANP). *J-P. Therrien, C.L. Tock, M. Ohyama, J.C. Vogel.* Dermatology Branch, NCI/NIH, Bethesda, MD, 20892-1908, USA.

Skin is a very attractive organ for targeted gene therapy to systemically deliver therapeutic proteins to treat a variety of disorders, including genetic anomalies. Skin is a renewable tissue, the ability to achieve long-term stable expression of a therapeutic gene requires targeting keratinocytes stem cells (KSC), but specific extracellular markers to isolate living KSC do not exist. We have therefore developed an *in vivo* system using a bicistronic retroviral vector expressing the desired therapeutic gene and a selectable marker (MDR) combined with topical colchicine treatment to select and enrich for cells expressing the vector. As a model to validate our system we have chosen ANP as a therapeutic gene to treat hypertension. Although studies have demonstrated that ANP infusion can decrease blood pressure in hypertensive patients and animal models, stable long-term expression of ANP from a targeted tissue such as skin will be required for clinical application. We have now constructed and produced a retroviral vector containing ANP and MDR genes and achieved viral titer 10^4-10^5 cfu/ml, similar to titers obtained in other studies using MDR in a bicistronic system. FACS analysis with anti-MDR antibody, shows that up to 32.5% and 18.6% of transduced-fibroblasts (Fb) or keratinocytes (Kc), respectively, express MDR. The more sensitive rhodamine exclusion assay demonstrated higher percentages of transduced-cells expressing a functional MDR in the plasma membrane, Fb (40.3%) and Kc (36.8%). We anticipate that colchicine selection will further increase the percentage of Kc expressing ANP, as well as ANP expression longevity. Human skin equivalent (HSE) expressing ANP/MDR has been engineered *in vitro*, with histology similar to normal human skin. Using an immuno-radiometric assay specific for ANP, bioengineered HSE were able to secrete significant levels of ANP, between 12 to 42 pg/ml, into the culture media. The genetically modified HSE is being grafted onto immunocompromised mice to determine the level of systemic human ANP achieved and the effect on murine blood pressure.

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Developing AAV-Mediated Gene Therapy For Acute Intermittent Porphyria: Vector Optimization Studies. *M. Yasuda, M. Domaradzki, D.F. Bishop, R.J. Desnick.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Acute Intermittent Porphyria (AIP), the most common hepatic porphyria, is an autosomal dominant disorder caused by the half-normal activity of Hydroxymethylbilane Synthase (HMBS). Clinically, AIP is manifested by life-threatening, acute neurological attacks that are provoked by various factors (e.g. cytochrome P450 inducing drugs, fasting, alcohol, and hormonal changes). Currently available therapy alleviates, but does not prevent the symptoms of the acute attacks. Thus, our goal is to develop a preventative therapy for AIP, using Adeno Associated Viral (AAV) vectors. For vector optimization, different combinations of liver-specific enhancers and promoters were evaluated for hepatic transgene expression levels. The murine HMBS cDNA was cloned into four expression vectors, each carrying a unique combination of liver-specific enhancers and promoters: HMBS-1 [human 1-microglobulin (1MG)/ 1-antityrpsin (1AT)], HMBS-2 [1MG/ human serum albumin (HSA)], HMBS-3 [human prothrombin (PT)/ HSA] and HMBS-4 [PT/ 1AT], and these HMBS constructs were transiently transfected into HepG2 cells. While all four constructs displayed increased HMBS activities compared with the mock transfection, HMBS-1 had the highest levels, with a ~9 fold increase over endogenous levels. Using the hydrodynamic technique, the HMBS constructs were then co-injected with a control luciferase construct into an AIP mouse model (Lindberg et al., Nat Genet 12: 195, 1996). 24 hours post-injection, the peak expression time for HMBS in vivo, livers were isolated from the mice and assessed for transgene expression. Again, the HMBS-1 construct displayed the highest levels, with a ~7 fold increase over the saline-treated mice. In conclusion, HMBS-1, carrying the 1MG enhancer and 1AT promoter, achieved the highest levels of hepatic HMBS expression, both in vitro and in vivo studies. Therefore, a rAAV-vector with these regulatory elements is being used to obtain rAAV, to evaluate the efficacy and safety of AAV-mediated gene therapy in the AIP mice.

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In Vivo Evaluation of Coagulation Factor VIII Variants for the treatment of Hemophilia A. *V. Cerullo[1], W.M. McCormack[1], R. Garcia[1], C. Clarke[1], S. Pipe[2], B. Lee[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI.

Hemophilia A is an X-linked bleeding disorder resulting from a deficiency of coagulation factor VIII (FVIII). FVIII expression after gene transfer is limited by unstable mRNA, interaction with endoplasmic reticulum (ER) chaperones, a requirement for facilitated ER to Golgi transport through interaction with the mannose-binding lectin LMAN1, and the instability of the activated form of FVIII (FVIIIa). Bioengineering of different variants of recombinant FVIII molecules by rational design can overcome these limitations. In this study we evaluated the in vivo efficacy of six different variants of recombinant FVIII. To this end, we generated six different helper dependent adenoviral vectors carrying human cDNA's regulated by the liver tissue restricted PEPCK promoter. To compare the short term efficacy and duration of hFVIII expression and infectivity of the different HDV hFVIII variants, FVIII deficient mice were treated with each vector at a dose of 5X1012 vp/kg via tail vein injection. Plasma was collected at baseline, two and four weeks post-injection and the FVIII biological activity was quantified by Coatest chromogenic bioassay. In this experiment, all vectors expressed functional FVIII at two weeks post-injection. FVIII activity varied from 2-8% in WT FVIII to up to 70% in other constructs. Functional FVIII activity dropped significantly in all vectors by 4 weeks except in the mice treated with an inactivation resistant rFVIII (HDV IR8 hFVIII) suggesting that this variant may exhibit an improved immunological profile. Interestingly, another construct containing the F309S substitution which decreases binding to the ER protein BiP to increase the rate of secretion of the FVIII variant, appears to significantly improve activity over WT hFVIII in vivo. By using these engineered FVIII variants, longer-lived protein with improved immunological profiles can be generated for gene transfer and protein therapy.

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Improved intracellular stability of multimeric hammerhead ribozymes. *D. Basel[1], B. Peace[2], Z. Lada[1], R.J. Wenstrup[2], M.W. Kilpatrick[1], P. Tsipouras[1].* 1) Gen&Dev Biol,UCHC, Farmington, CT; 2) CCHRF, Cincinnati, OH.

Hammerhead ribozymes are small catalytic RNA molecules that can be engineered to cleave RNA in a sequence-dependent manner. In earlier studies we have shown that ribozyme can efficiently abolish the mutant transcript in a cellular model of Osteogenesis Imperfecta. Additionally, we have shown that ribozyme multimers capable of cis-cleaving to catalytically active monomeric subunits have greater intracellular activity than their constituent monomers. Interestingly, extending the 3 tail of a monomeric ribozyme resulted in both increased stability and efficacy. We postulated, therefore, that the greater efficacy of multimeric ribozymes in the cell might result from the larger ribozyme construct being more stable than the smaller monomer. To test this hypothesis, cell lines expressing a ribozyme monomer, a cis-cleaving ribozyme multimer and a non-cis cleaving ribozyme multimer were established. Total RNA preparations from cells expressing the monomeric ribozyme and cis-cleaving multimeric ribozyme were analyzed for the presence of ribozyme by ribonuclease protection assay and slot-blot analysis. Initial data indicates that the half-life of the ribozyme monomer is approximately one third that of the cis-cleaving multimer. This supports our hypothesis that the larger transcript is more resistant to intracellular degradation. The second phase of this work is to compare the cis- and non cis-cleaving ribozyme multimers, which both contain six copies of the active ribozyme, and differ only in their ability to cleave or not-cleave, to monomeric sub-units. These ribozymes specifically cleave their mutant Col1A1 mRNA target in a test tube and, as expected, the cis-cleaving multimer self cleaves to its monomeric subunit while the non cis-cleaving multimer remains a single molecule. Analysis of cell lines that stably express each of these two ribozyme constructs will both compare the half life of cis-cleaving multimer to that of a non cis-cleaving multimer and determine their relative intracellular target cleaving efficacies. This data may lead to new ways of designing ribozymes for intracellular expression and subsequent application in targeted gene therapies.

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Inability of an insulator element to prevent insertional activation of oncogenes and tumorigenesis. *J. Lenz, K. Mitra.* Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY.

Retrovirus gene therapy vectors have proven successful in treating human inherited disorders. However, trials of murine gammaretrovirus (MLV) vectors in humans have encountered the complication of insertional activation of host oncogenes by the viral vectors leading to tumorigenesis. One strategy that has been widely proposed to prevent this complication and improve the safety of gene therapy vectors is to use insulator elements to prevent transcriptional enhancers in the vector from activating the promoters of adjacent oncogenes. However, this has not been tested directly. We analyzed the ability of the chicken -globin HS4 insulator (cHS4) element to prevent insertional activation of oncogenes and T-cell lymphomagenesis by an MLV. MLVs have been extensively used for genome-based, high throughput molecular identification of oncogenes (http://rtcgd.ncifcrf.gov/). Enhancer activation of oncogenes, usually from distances >10 kbp, is by far the most frequent mechanism of tumorigenesis by these retroviruses. We inserted the cHS4 element into the LTR of a T-cell lymphomagenic, replication-competent MLV. The element was stable within the viral genome for multiple rounds of viral replication in cultured cells. Infection of mice induced a modest, though statistically significant, slowing of the appearance of lymphomas. We then applied a genomics-based approach to determine the insertion sites of MLVs in the genomes of the tumor cells. This analysis showed that inserted proviruses were able to activate flanking oncogenes (*Myc, Rras2, Rasgrp1, Ccnd3, Kras, Runx1, Gfi1, Fos, Pim1*) by an enhancer mechanism even when the cHS4 element was positioned between the viral enhancer and the promoter of the mouse oncogene. Moreover, the percentage of tumors with such activation events was the same with either the insulated or the wild type virus. Thus the cHS4 element did not prevent insertional activation of oncogenes. These results mean that the use of insulators to block oncogene activation by inserting gene therapy vectors is not a simple issue. Rather it will require careful experimentation to devise an effective approach.

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Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy. *R. Allikmets[1,2], J. Kong[1], S.R. Kim[1], K. Binley[3], S. Naylor[3], P. Leboulch[4], J.R. Sparrow[1,2], P. Gouras[1].* 1) Dept Ophthalmology, Columbia Univ, New York, NY; 2) Dept Pathology & Cell Biology, Columbia Univ, New York, NY; 3) Oxford BioMedica, Oxford, UK; 4) Genetix Pharmaceuticals, Cambridge, MA.

Stargardt disease (STGD) is a macular dystrophy caused by mutations in the *ABCA4 (ABCR)* gene. The most recognized phenotypic feature of STGD patients and the mouse model, the Abca4$^{-/-}$ mice, is lipofuscin (A2E) accumulation in retinal pigment epithelium (RPE), considered to be the cause of RPE cell death at late stages of the disease. Here, we tested whether delivery of the normal (wt) human ABCA4 gene to the subretinal space of the Abca4$^{-/-}$ mice via lentiviral vectors would correct the disease phenotype, i.e., reduce A2E accumulation. Human immunodeficiency virus (HIV) and equine infectious anemia virus (EIAV)-derived lentiviral vectors were constructed, with the human *ABCA4* gene, or the reporter gene *LacZ*, under the control of constitutive (CMV, EF1) or photoreceptor-specific (Rho) promoters. Abca4$^{-/-}$ mice were injected subretinally with 1-3x10^5 IU of each virus at P4-5 in one eye; the mock-injected contralateral eye served as a control. Mice were sacrificed at 4, 6, and 8 months after injection and the A2E content was determined by HPLC. Subretinal injections of ~10^5 IU of the HIV-LacZ effectively transduced RPE cells, but less effectively (<5%) photoreceptors (PR). In comparison, the same amounts of EIAV-LacZ vectors were more effective in transducing PR in both mice (>30%) and rabbits (100%). Most importantly, a single subretinal injection of 3x10^5 IU of HIV-EF1-ABCA4 to Abca4$^{-/-}$ mice practically eliminated A2E accumulation compared to mock-injected controls. Treated eyes of Abca4$^{-/-}$ mice accumulated 4-6 pmol/eye of A2E 4-8 months after treatment (amounts similar to wt controls), while mock-treated eyes had 6-8 times more A2E (22-35 pmol/eye). In summary, the excessive A2E accumulation in the mouse model of STGD disease was eliminated by lentivirus-based gene therapy, suggesting that lentiviral gene therapy is a potentially efficient tool for treating *ABCA4*-associated diseases.

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Evaluation system for siRNA duplexes conferring allele-specific gene silencing. *Y. Ohnishi[1,2], K. Tokunaga[1], K. Kaneko[2], H. Hohjoh[2].* 1) Depertment of Human Genetics, Graduate School of Medicine, The university of Tokyo, Tokyo, Japan; 2) National Institute of Neurosience, NCNP, Tokyo, Japan.

We have developed an easy evaluation system for small interfering RNA (siRNA) duplexes conferring allele-specific gene silencing, i.e., allele-specific RNA interference (RNAi). The system depends on heterozygous reporter plasmids encoding the Photinus and Renilla luciferase genes, whose 3'f untranslated regions carry allelic sequences of interest; e.g., one is mutant allelic sequence, and the other is corresponding wild-type sequence. In this study, we chose the Swedish- and London-type amyloid precursor protein (APP) mutants related to familial Alzheimer'fs disease as model mutant alleles, and effect of the siRNA duplexes designed against the mutants on allele-specific gene silencing and also off-target gene silencing against the wild-type APP allele were simultaneously determined under heterozygous condition generated by cotransfeciton of the reporter alleles and siRNA duplexes into cultured mammalian cells. The siRNA duplexes conferring allele-specific gene silencing consistently reduced the amounts of the mutant APP and also beta-amyloid peptide in'@heterozygous Cos-7 cells with the Swedish- and wild-type APP expression plasmids. Therefore, the system presented here could allow us to design and/or select most suitable siRNA duplexes conferring allele-specific gene silencing to mutant alleles before using them in vivo or in therapeutic trials.

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Towards a first proof-of-concept clinical study on antisense-induced exon skipping in Duchenne Muscular Dystrophy. *J. Van Deutekom[1], A. Aartsma-Rus[1], A. Janson[1], C. de Winter[1], H. Heemskerk[1], J. Verschuuren[2], G. Platenburg[3], GJ. van Ommen[1].* 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept Neurology, Leiden Univ Medical Ctr, Leiden, Netherlands; 3) Prosensa B.V., Leiden, Netherlands.

The promise of antisense oligonucleotides (AONs) as small molecule drugs for Duchenne muscular dystrophy (DMD) has unquestionably been established over the last few years. We have successfully applied human sequence-specific AONs to induce the skipping of specific exons during the splicing of the gene's pre-mRNA in cultured muscle cells from a series of DMD patients with different mutations. This exon skipping restored the open reading frame of the mutated DMD transcripts, and thus allowed the synthesis of internally truncated, but largely functional dystrophins, as found in patients with the less severe Becker muscular dystrophy (BMD). With dystrophin expression generally detected in up to 90% of treated muscle cells, the efficiency of this therapy in vitro is remarkably high. In vivo proof-of-principle for therapeutic exon skipping has been obtained in mdx mice, a DMD mouse model carrying a nonsense mutation in exon 23. Intramuscular injections of AONs that induce exon 23 skipping, restored dystrophin in up to 20% of fibers which significantly improved local muscle function. Encouraged by these promising pre-clinical results, we have set up a first proof-of-concept clinical study. For the initial assessment of safety, tolerability and efficacy, we have chosen for a local, intramuscular administration of an exon 51 skipping AON (h51AON23) in the tibialis anterior muscles of 4 to 6 DMD patients aged between 8-16 years. The clinical GMP batch of h51AON23, a 2'OMePS modified RNA molecule, has been produced and validated by reproducible dose-response curves in human muscle cell cultures. The toxicology program under full GLP compliance is in progress, and the clinical protocol has recently been optimized in rabbits. Upon final approval by the Dutch Medical Ethics Committee, the study will start at the end of this year 2005.

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Development of Survival Motor Neuron Recombinant Adeno-Associated Virus Vectors. *M. Shababi[1], T. Baughan[1,2], G. Tullis[2], C. Lorson[1].* 1) Department of Veterinary Pathobiology,; 2) Department of Molecular Microbiology and Immunology. Life Sciences Center, University of Missouri,Columbia, MO, 65211.

Spinal muscular atrophy (SMA), an autosomal recessive neurodegenerative disease, is caused by loss of -motor neurons in the spinal cord. SMA is caused by the homozygous loss of the telomeric SMN1 (survival motor neuron 1) gene, that leads to extremely reduced levels of the functional SMN protein. A highly homologous centromeric copy gene (SMN2) exists in almost all SMA patients, but fails to complement SMN1 loss due to its defective splicing pattern. A single nucleotide transition in the 5 end of the SMN2 exon 7 alters splicing and gives rise to the mRNA lacking exon 7 and encodes a biochemically defective and unstable protein that is unable to protect against SMA. The fact that the SMN2 gene has the capacity to encode an identical protein to SMN1 makes this disease very amenable to therapeutic interventions. One logical gene therapy approach is to correct the splicing defect of the SMN2 gene. To accomplish this goal, we have constructed short RNAs designed to modulate SMN2 splicing that are delivered via recombinant adeno-associated virus vectors. Our results demonstrate that the viral-based delivery of these RNA molecules into SMA patient fibroblasts stimulates increased levels of the SMN full-length protein and represents a mechanism to modulate disease-causing splicing defects.

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Ex vivo gene therapy for recessive dystrophic epidermolysis bullosa using safe retroviral vectors expressing type VII collagen under the control of its promoter. *M. Titeux[1], M.A. Zanta-Boussif[2], A. Rochat[3], A. Décha[1], L. Tonasso[1], Y. Barrandon[3], O. Danos[2], A. Hovnanian[1,4].* 1) U563-CPTP, INSERM, CHU Purpan, Toulouse, France; 2) Généthon III, Evry, France; 3) EPFL/CHUV, Lausanne, Swiss; 4) Service de Génétique, CHU Purpan, Toulouse, France.

Our goal is to develop the essential preclinical steps for ex vivo gene therapy for recessive dystrophic epidermolysis bullosa (RDEB, OMIM #22600). RDEB is caused by loss of function mutations in the collagen VII gene (COL7A1) encoding anchoring fibrils. It is one of the most severe genodermatoses in children and adults. The patients suffer since birth from skin blistering, and from severe local and systemic complications resulting in poor prognosis. We lack a specific treatment for RDEB, but ex vivo gene transfer to epidermal stem cells shows a therapeutic potential. The chosen approach consists of transferring the COL7A1 cDNA under the control of its promoter, using safe (SIN, Self Inactivating) retroviral and lentiviral vectors into epidermal stem cells and dermal fibroblasts. These vectors have no selection marker, they are self inactivating (reducing the risk of oncogenic events), and they provide a physiological regulation of COL7A1 expression. We have used these vectors to genetically correct primary RDEB keratinocytes and fibroblasts ex vivo. The transduced keratinocytes are able to form fully differentiated epithelia using a skin equivalent system made of a human plasma derived fibrin gel including corrected RDEB fibroblasts. This permits a fully autologous graft onto patients. We have demonstrated long term in vivo expression of recombinant type VII collagen and the formation of anchoring fibrils in these genetically corrected skin equivalents after grafting onto nude mice. The safe vectors, the tissue specificity of type VII collagen expression and the autologous skin equivalent system used constitute key assets for a clinical application. Finally, using this vector, we aim to graft skin equivalents made of genetically corrected autologous keratinocytes and fibroblasts onto the most severely affected skin areas of patients.

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Enzyme Replacement Therapy for Fabry Disease: A Safe, Shorter Infusion Protocol. *M. Banikazemi, R.J. Desnick.*
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Fabry disease is an X-linked disorder of glycosphingolipid metabolism due to the deficient activity of the lysosomal enzyme -galactosidase A. Clinical trials of enzyme replacement therapy with Fabrazyme (Genzyme Corp,1mg/kg every 2 weeks) have shown that the enzyme safely and effectively cleared the accumulated globotriaosylceramide from the plasma, heart, kidney, liver, and skin (Eng et al., *Am J Hum Genet* 68 :711, 2001 and *New Engl J Med* 345:9, 2001). A recent phase 4 double-blinded, placebo-controlled trial demonstrated clinical benefit in slowing the progression of the renal, cardiac, and cerebrovascular complications in patients with advanced renal disease, providing further clinical evidence of the effectiveness of ERT in this disease. During the clinical trials, in order to keep the double-blind, enzyme infusion rates were prolonged (15 mg/hr regardless of weight) to minimize the incidence of infusion associated fevers and chills which occurred in some patients. For the clinical trials, the infusion solution was prepared by reconstitution of the Fabrazyme dose with 0.9% sodium chloride in total volume of 500 mL. Here, we report a safe and shorter infusion protocol using a ramping protocol that increases the infusion rate from 0.1 mg/kg body weight/hr to a maximum of 0.7 mg/kg/hr in four ramp steps. Fabrazyme (1 mg/kg) is prepared in 100 mL of 0.9% sodium chloride for injection, and then the solution is infused at a rate of 0.1 mg/kg/hr for 15 min (step 1). The rate is then increased by 0.2 mg/kg/hr every 15 to 30 minutes as tolerated (steps 2 & 3). The maximum rate at the final ramp step 4 is 0.7 mg/kg/hr. The total infusion time is under 2 hours. For example, in the clinical trial protocol, a 70 kg patient would be initially infused at a rate of 15 mg/hr for 4.67 hour. With the ramping protocol, this time would be reduced to 1.9 hr. If the infusion is well tolerated the rate can be increased so that the total infusion is accomplished in 90 min. This shorter infusion protocol is particularly well tolerated, and the time saving is appreciated by the patient and the medical staff, whether administered in the clinic or at home.

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Expression in blood cells may contribute to biochemical and pathological improvements after neonatal intravenous gene therapy for mucopolysaccharidosis VII in dogs. *M. Haskins[1], B. Wang[2], T. O'Malley[1], L. Xu[2], P. Wang[1], P. O'Donnell[1], N.M. Ellinwood[3], K.P. Ponder[2].* 1) Dept Pathobiology, Sch Vet Med, Univ Pennsylvania, Philadelphia, PA; 2) Dept Int Med & Biochem & Biophys, Sch Med, Washington Univ, St. Louis, MO; 3) Dept Animal Sci, Iowa State Univ, Ames, IA.

Mucopolysaccharidosis VII (MPS VII) is a lysosomal storage disease due to deficient activity in beta-glucuronidase (GUSB) that results in the accumulation of glycosaminoglycans (GAGs) throughout the body. We have previously reported that neonatal intravenous injection of 3x10E9 transducing units (TU)/kg of a gamma retroviral vector (RV) expressing canine GUSB resulted in transduction of hepatocytes, an average serum with mannose 6-phosphate modified GUSB activity of 195+/-36 U/ml (SEM), which is 73% of normal, with reduction in clinical disease manifestations in the heart, bone, and eye. However, it was unclear if liver was the only site of expression, and the effect upon other organs was not assessed. At various time points including at 4.5 years, we demonstrated that blood cells from these RV-treated MPS VII dogs had 1 copy of retroviral vector per 100 cells, and expression of the RV RNA was at 2% of the level found in liver. Expression of GUSB in blood cells may, therefore, synergize with uptake of GUSB from blood to reduce storage in organs. At 6 months, two of the RV-treated dogs had marked biochemical and pathological evidence of reduction in storage in liver, thymus, spleen, small intestine, and lung, although the effect was only partial in kidney tubules. The brain had 3% of normal GUSB activity, and biochemical and pathological evidence of reduction in storage in neurons and other cell types. Secretion of the enzyme into blood by hepatocytes and other tissues, and expression in blood cells that migrate into organs, may contribute to correction of disease. Thus, this neonatal gene therapy approach was effective and might be used in humans if it proves to be safe.

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Oral miglustat in adult and pediatric patients with Niemann-Pick type C (NPC) disease: rationale and methodology of a clinical study. *M.C. Patterson[1], D. Vecchio[1], H. Prady[2], N. Ait-Aissa[3], L. Abel[4], E. Wraith[2].* 1) Dept of Neurology, Columbia University, New York, USA; 2) Royal Manchester Children's Hospital, Manchester, UK; 3) Actelion Pharmaceuticals Ltd, Switzerland; 4) Dept of Optometry & Vision Sciences, University of Melbourne, Melbourne, Australia.

NPC is a neurodegenerative disease that is characterized by impaired intracellular trafficking and sequestration of macromolecules. In addition to unesterified cholesterol, NPC1 protein-deficient cells accumulate gangliosides and other glycosphingolipids. Miglustat is a reversible inhibitor of glucosylceramide synthase, a key enzyme in the synthesis of glycosphingolipids; miglustat crosses the blood-brain barrier. Studies in animal models of NPC disease showed that miglustat delayed the onset of neurological dysfunction, increased life span (in mouse) and reduced ganglioside accumulation and neuropathological changes. In this first clinical study of this therapeutic approach in NPC disease, 41 patients (29 adults; 12 children <12 yrs) with abnormal cholesterol esterification and filipin staining were enrolled. Adult patients were randomized 2:1 to receive miglustat, 200 mg t.i.d., or standard care for 1 year. All children received miglustat, with dosage adjusted for body surface area. At the 1-year time point, adult patients could elect to enter the extension study with active treatment for an additional year. NPC is a very heterogeneous disease, and selection of the study primary endpoint of horizontal saccadic eye movement velocity (SEMV) is based on the established correlation between the severity of supranuclear gaze palsy in NPC and disease progression. Secondary endpoints include neurological examination, tremor and swallowing assessments, MMSE, Purdue Peg Board, liver and spleen organ volumes, chitotriosidase, QoL (SF-36 and CHQ-PF50), and vertical SEMV. The pediatric substudy has simplified and corresponding age-appropriate neurological and neuropsychological assessments. Miglustat is the first substrate-inhibiting drug to be investigated in NPC patients; evaluation of its safety and efficacy will be based on the comprehensive set of outcomes described.

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A clinical study to assess the safety and efficacy of oral miglustat in late-onset Tay-Sachs (LOTS) disease: baseline demographics and disease manifestations. *B. Shapiro[1], G.M. Pastores[2], E.H. Kolodny[2].* 1) Department of Neurology, Cleveland University Hospitals, Cleveland, USA; 2) Neurogenetics Unit, Department of Neurology and Pediatrics, NYU School of Medicine, New York, USA.

Tay-Sachs disease, an autosomal recessive lysosomal storage disorder caused by deficiency of -hexosaminidase A, is associated with the accumulation of G_{M2}-ganglioside in the CNS. In the adult, late-onset variant, individuals develop slurred speech, muscle weakness, tremors, unsteady gait and mental illness, particularly psychoses and depression. We describe baseline (BL) demographics and disease manifestations of the largest cohort of LOTS patients studied systematically, enrolled in a study investigating substrate reduction therapy (SRT) with oral miglustat (currently approved for adult mild-to-moderate type 1 Gaucher disease). In preclinical studies, miglustat prevented the storage of G_{M2}-ganglioside within the CNS [Zervas et al. Curr Biol 2001;11:1283-7]. **Methods**: Patients 18 years were enrolled in a study to assess the safety and efficacy of miglustat, 200 mg t.i.d., vs standard clinical care. Comprehensive BL evaluations included quantitative isometric muscle strength, speech, gait and balance assessments. **Results**: Thirty patients were enrolled (20 males). Mean age was 38.6 yrs. The most common disease manifestations noted at BL were muscle weakness (97%), tremor (93%), dysarthria (87%), ataxia (83%), ambulation difficulties (80%), dysdiadochokinesis (80%), muscle cramps (77%), dysmetria (73%), swallowing difficulties (53%), depression (40%), learning difficulties (33%), psychosis (33%) and bipolar disease (27%). Tinetti scale scores at BL were 7.8 for balance (maximum score 16), and 7.2 for gait (maximum score 12). **Conclusions**: LOTS is a progressive neurodegenerative disorder that is currently untreatable except possibly by SRT. Our study revealed wide heterogeneity in BL disease characteristics. As a model for clinical trials in small patient populations, our findings in LOTS patients point to several potential confounders in assessment of treatment effect, particularly when the period of observation is short.

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ICAM-1 targeting enhances delivery of active acid sphingomyelinase in mice. *S. Muro[1], R. Dhami[3], J. Leferovich[1], V. Muzykantov[1, 2], E. Schuchman[3].* 1) Institute for Environmental Medicine and; 2) Dept. Pharmacology, University of Pennsylvania Medical School, Philadelphia, PA; 3) Dept. Human Genetics, Mount Sinai School of Medicine, New York, NY.

An inherited deficiency of acid sphingomyelinase activity (ASM) causes the lysosomal storage disorder (LSD), Types A and B Niemann-Pick Disease (NPD), affecting multiple organ systems (brain, liver, spleen, and lung). Ineffective clathrin-mediated internalization of recombinant ASM by NPD cells, which is mediated by receptors recognizing enzyme sugar-residues (mannose-6-phosphate), may hinder efficacy of enzyme replacement therapy (ERT) for NPD. We previously showed that targeting recombinant ASM to the surface glycoprotein InterCellular Adhesion Molecule (ICAM)-1 by anti-ICAM nanoparticles bypasses glycosylation-mediated binding and clathrin-mediated internalization, improving enzyme delivery to lysosomes. To evaluate the efficacy of this delivery system in vivo, we determined the pharmacokinetics and biodistribution of free ^{125}I-ASM vs. ^{125}I-ASM coupled to FITC-labeled anti-ICAM nanoparticles administered IV in C57Bl/6 mice. Both ASM and anti-ICAM/ASM nanoparticles were rapidly cleared from the circulation (38.23.2 % vs. 21.45.1 % of the injected dose, ID, by 1 min; and 17.67.8 % vs. 2.60.3 % ID by 15 min post-injection). However, ASM coupled to anti-ICAM nanoparticles showed greater levels vs. free ASM in all organs analyzed 30 min post-injection (except kidney, 0.85 fold), including heart (1.6 fold), brain (1.7 fold), liver (2.5 fold), spleen (5.3 fold), and lung (25 fold). Fluorescence microcopy confirmed the presence of ASM-loaded, FITC-labeled anti-ICAM nanoparticles in these organs. Injection of anti-ICAM/ASM nanoparticles in ASM knockout mice, a model for NPD, increased ASM activity over the endogenous levels in heart (1.5 fold), brain (1.5 fold), kidney (5.0 fold), liver (17.1 fold), lung (19.7 fold), and spleen (59.2 fold). Therefore, targeting to ICAM-1 enhances delivery of active ASM to primary pathological sites, representing a promising strategy to optimize ERT for NPD and other LSD. Funding: AHA 0435181N (SM), NIH HD 28607 (ES) and HL/GM 71175-01 (VM), and DOD PR 012262 (VM).

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Dystrophin Expression in Myogenic Converted Fibroblasts from Duchenne Muscular Dystrophy using Antisense RNA/ENA Chimera. *Z. Zhang, Y. Takeshima, M. Yagi, M. Matsuo.* Department of Pediatrics, Kobe University Graduate School of Medicidne, Kobe, Japan.

The use of antisense oligonucleotides to induce exon skipping leading to generation of an in-frame dystrophin mRNA could be of benefit in around 70% of Duchenne muscular dystrophy (DMD) patients. We have previously reported that a novel antisense RNA/Ethylene-bridged nucleic acid (ENATM, Sankyo Lifetech Co., Ltd., Tokyo, Japan) chimera was 40 times as effective as a conventional phosphorothioate oligonucleotide in inducing exon 19 skipping of dystrophin gene in cultured muscle cells. To further enlarge the therapeutic application of this chemical, we here designed specific RNA/ENA chimera to target exons which located in deletion hotspots of dystrophin gene. Different with pilot experiments in human muscle cells, we use fibroblasts from two DMD patients carring different deletions as a alternative source of muscle cells. Fibroblasts were induced to differentiate to myogenic lineage by AdMyoD, and exposed to RNA/ENA chimera. The exon skipping and dystrophin expression were monitored by RT-PCR and dystrophin staining. In all cases, the targeted exon was specifically skipped at relatively high levels, which induced the synthesis of significant levels of dystrophin. These results document that myoD-forced myogenesis provide a powerful tool to study the efficiency of the antisense based therapy, and that our new RNA/ENA chimera could induce skipping of dystrophin exons located in deletion hot spots, thereby making dystrophin expression possible in a broader spectrum of DMD cases.

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Results of a 22 year clinical trial: Acute, adjunctive pharmacological treatment of hyperammonemic episodes in patients with urea cycle disorders (UCD). *S.E. Gargosky[1], M. Summar[2].* 1) Research & Development, Ucyclyd Pharma, Scottsdale, AZ; 2) Vanderbilt University Medical Center DD-2205 Medical Center North Nashville, TN 37232.

UCDs are a rare, ultra-orphan group of diseases. To amass a database comprising 1045 with episodes and over 300 patients had been an enormous undertaking. This historical database provides demographics, survival as well as clinical history of these patients treated with AMMONUL (sodium phenylacetate/sodium benzoate) injection 10%/10%. On admission to the hospital, patients with hyperammonemia or a potential UCD were treated with a bolus dose of 0.25 g/kg (or 5.5 g/m2) AMMONUL over a period of 90 minutes to 6 hours, depending on the specific UCD. Infusions also contained Arginine. After completion of the bolus dose, maintenance infusions of the same dose over 24 hours were to be continued until the patient was no longer hyperammonemic or oral therapy could be tolerated. Episodes in this report occurred at 115 hospitals/316 patients/1045 episodes. The mean (SD)number of episodes per patient was 3.3 (6.18) and ranged from 1 to 77. Of the 316 patients, 185 experienced 1 episode, 53 had 5 or more episodes, 48 had 2 episodes, and 30 had 3 or 4 episodes. The mean length of hospitalization per episode was 9.4 (12.97) days and ranged from 1 to 171 days. 76%, patients were hospitalized for fewer than 10 days. The patient population was 51% male and 49% female. The mean (SD) age for all episodes was 8.5 (7.44) years, and ranged from 0 to 53 years. For all patients, the mean age at the first episode was 6.2 (8.54) years, and ranged from 0 to 53 years. Survival rates at the end of hyperammonemic episodes were substantially improved after treatment with AMMONUL, compared with historical controls. In 94% of the episodes, the patient was alive at discharge. In patients responding to therapy, mean ammonia levels decreased significantly within four hours of initiation of AMMONUL therapy and were maintained. Thus, the wide spread, rare nature and absence of regionalization makes this database a unique source for examining this patient population.

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Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. *B.C. Capell[1], R. Varga[1], M.R. Erdos[1], M. Eriksson[2], J.P. Madigan[3], J.J. Fiordalisi[3], L.B. Gordon[1], C.J. Der[3], A.D. Cox[3], F.S. Collins[1].* 1) NHGRI, NIH, Bethesda, MD; 2) Karolinska Institutet, Huddinge, Sweden; 3) UNC-Chapel Hill, Chapel Hill, NC.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by dramatic premature aging and accelerated cardiovascular disease. HGPS is almost always caused by a *de novo* point mutation in the lamin A (*LMNA*) gene that activates a cryptic splice donor site, producing a truncated mutant protein, termed progerin. Wild-type prelamin A is anchored to the nuclear envelope by a modification of the C-terminal CAAX motif by a farnesyl isoprenoid lipid. Cleavage of the terminal 15 amino acids and the farnesyl group releases mature lamin A from this tether. In contrast, this cleavage site is deleted in progerin. We hypothesized that retention of the farnesyl group causes progerin to become permanently anchored in the nuclear membrane, disrupting proper nuclear scaffolding and causing the characteristic nuclear blebbing seen in HGPS cells. We further hypothesized that blocking farnesylation would decrease progerin toxicity. To test this, the C-terminal CSIM sequence in progerin was mutated to SSIM, a sequence that cannot be farnesylated. SSIM progerin relocalized from the nuclear periphery into nucleoplasmic aggregates and produced no nuclear blebbing. Further, blocking farnesylation of authentic progerin in transiently transfected HeLa, HEK-293, and NIH-3T3 cells with farnesyltransferase inhibitors (FTIs) restored normal nuclear architecture. Likewise, treatment of both early and late passage human HGPS fibroblasts with FTIs resulted in significant reductions in nuclear blebbing. To test this therapeutic approach *in vivo*, we are now administering oral FTIs to a transgenic mouse model of HGPS. This model was created by recombineering a 164 kb human BAC containing the *LMNA* gene to incorporate the 1824C>T (G608G) mutation. Mice from a stable transgenic line express the mutant *LMNA* RNA and protein (progerin) products, and display a vascular phenotype strikingly similar to the atherosclerotic disease seen in patients with HGPS.

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Pharmacological reactivation of the FMR1 gene: comparative effect of 5-aza-2-deoxycytidine and valproic acid.
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To clarify the mechanisms that suppress the activity of the mutant FMR1 gene in the fragile X syndrome and the conditions that may permit its reactivation, we investigated the acetylation and methylation status of three different regions of the FMR1 gene (promoter, exon 1 and exon 16) of three fragile X cell lines, before and after 5-aza-2-deoxycytidine (5-azadC) reactivating treatment. Basal levels of histone acetylation and H3-K4 methylation were much higher in transcriptionally active wild-type controls than in inactive fragile X cell lines. Treatment of fragile X cell lines with 5-azadC induces a decrease of H3-K9 methylation, an increase of H3 and H4 acetylation and of H3-K4 methylation. Our experiments indicate that, whereas histone acetylation plays an ancillary role, H3-K4 methylation and DNA demethylation are the main epigenetic switches activating FMR1 transcription. In an effort to identify other reactivating drugs, we tested the effect of valproic acid (VPA), widely used in humans as anticonvulsant, on the epigenetic status of FMR1 in fragile X cell lines. Recent data suggest that VPA inhibits histone deacetylases (HDACs) and/or induces DNA demethylation. To test VPA toxicity, we treated cells with increasing drug concentrations and found that the best reactivating effect is obtained with three days treatment at 2mM concentration. VPA shows a modest but rapid reactivating activity, compared to 5-azadC. The comparison between these two drugs effects suggests that VPA, in contrast to 5-azadC, may work in a cell-cycle independent fashion. This work was supported by grant GGP030202 from Fondazione Telethon to G.N.

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Use of Oral Citrulline as a Nitric Oxid Precursor in the Treatment of Post Cardiac Surgery Related Pulmonary Hypertension in Children. *M. Summar[1,2], H. Smith[2], G. Rice[1,2], J. Canter[1], R. Barr[1,2].* 1) Ctr Human Genetic Res, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Department of Pediatric, Vanderbilt Univ Medical Ctr, Nashville, TN.

Pulmonary hypertension can significantly complicate the perioperative course of children undergoing surgical correction of their congenital heart lesions. NO affects pulmonary vascular tone and is produced by nitric oxide synthase from arginine and its urea cycle precursor citrulline. We have shown that cardiac surgery significantly decreases concentrations of both citrulline and arginine. We hypothesize that addition of citrulline may promote better nitric oxide production with a subsequent reduction in the incidence of postoperative pulmonary hypertension. Study Design This was a randomized, placebo controlled, double-blinded study. Forty infants/children, undergoing cardiopulmonary bypass and at risk for pulmonary hypertension, received five doses (1.9 g/m2 /dose) of either citrulline or placebo, administered immediate preoperatively, immediate postoperatively, then every 12 hours for three doses. Plasma citrulline concentrations were measured at five time points. Secondary outcome measurements of systemic blood pressure, plasma arginine concentration, and presence/absence of pulmonary hypertension were collected. Results Median citrulline concentrations were significantly higher in the oral citrulline group compared with placebo immediately following bypass (36 umol/L vs 26 umol/L, P=0.012) and at 12-hours postoperatively (37 umol/L vs 20 umol/L, P=0.015). Mean plasma arginine concentrations were significantly higher in the oral citrulline group compared with placebo by 12-hours postoperatively (36 umol/L vs 23 umol/L, P=0.037). Mean systemic blood pressure during the 48-hour study period did not differ between groups (P=0.53). Nine patients developed postoperative pulmonary hypertension, all of whom had plasma citrulline concentrations less than the median concentration (37 umol/L) obtained with oral citrulline supplementation (P=0.036). Conclusions Perioperative oral citrulline administration may be a safe and effective way to reduce postoperative pulmonary hypertension.

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Fabry Disease in Females: Effects of Enzyme Replacement Therapy with Agalsidase Alfa. *F. Baehner[1], P. Deegan[2], D. Hughes[3], M. Barba[4], C. Kampmann[1], M. Beck[5].* 1) Dept. Pediatric Cardiology, Univ. Children's Hospital, Mainz, Germany; 2) Addenbrooke's Hospital, Cambridge, UK; 3) Royal Free & University College Medical School, London, UK; 4) Complejo Hospitalario de Albacete, Spain; 5) Metabolic Devision, Univ. Children's Hospital, Mainz, Germany.

Introduction: Fabry disease is an X-linked glycosphingolipid storage disorder caused by a lysosomal deficiency of -galactosidase A. Progressive accumulation of the substrate globotriaosylceramide in cells throughout the body leads to major organ failure and premature death. Studies have reported the high prevalence of disabling clinical features in heterozygous females, but little is known of the effects of enzyme replacement therapy (ERT) in female patients. Aims: This study examines the safety and efficacy of ERT in a cohort of 358 female patients with Fabry disease. 160 received treatment with agalsidase alfa. Methods: FOS - the Fabry Outcome Survey is a European outcomes database for all patients with Fabry disease who are receiving, or are candidates for, ERT with agalsidase alfa (Replagal). We analyzed this data to evaluate the safety and efficacy of agalsidase alfa administered intravenously to female patients with Fabry Disease. Design: The effects of ERT with agalsidase alfa on renal function (assessed by estimated glomerular filtration rate), heart size (assessed by echocardiography), heart function (Midwall fractional shortening), pain (assessed by the Brief Pain Inventory) and quality of life (QoL) (assessed by the European Quality of Life Questionnaire EQ-5D) were analyzed in a cohort of 160 patients. Results: Similar to males with Fabry disease ERT in females is well tolerated and safe. It reduces pain, stabilises renal function, improves QoL heart function and reduces heart size. Thus ERT is likely to alter the natural history of the disease in females.

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